9 BIOMOLECULES

A cell is made up of over 5000 chemicals. The structure and function of different cell constituents are an interplay of their constituent chemicals, their arrangement and properties.

The Cellular Pool

Biochemistry is the science dealing with chemicals and physico-chemical reactions found in living organisms and their life processes. Chemicals or molecules present in the living organisms are known as **biomolecules**. The collection or sum total of different types of biomolecules, compounds and ions present in a cell is called the **cellular pool**. The cellular pool has two phases, aqueous and non-aqueous. Aqueous phase contains chemicals dispersed in water forming either **true solution** (homogeneous, particle size 1 nm or less) or **colloidal solution** (heterogeneous, particle size 1–100 nm). Substances that form true solution are known as **crystalloids** while the ones that produce colloidal solution are called **colloids**. Non-aqueous phase contains chemicals deposited in various structures like chromatin, cell membrane and cell wall. The chemical analysis of living and nonliving matter, (earth is crust) reveal that both are made up of same types of elements but the relative abundance of C and H with respect to other elements is higher in any living organism than in nonliving matter or earth's crust (Table 9.1).

Table 9.1. A comparison of elements present in non-living and living matter

Element	% weight of	
	Earth's crust (non living matter)	Human body (living matter)
Hydrogen	0.14	0.5
Carbon	0.03	18.5
Oxygen	46.6	65.0
Nitrogen	very little	3.3
Sulphur	0.03	0.3
Sodium	2.8	0.2
Calcium	3.6	1.5
Magnesium	2.1	0.1
Silicon	27.7	negligible

Biomolecules are of two types, inorganic and organic.

(i) Inorganic constituents of cellular pool are minerals, gases and water. Organic constituents are carbohydrates, lipids, amino acids, proteins, enzymes, nucleotides, nucleic acids, vitamins, etc. (ii) Inorganic substances do not contain carbon alongwith hydrogen. Organic molecules possess carbon and hydrogen. (iii) Minerals occur in the form of salts ions (table 9.2). Organic materials occur both in aqueous and nonaqueous phases.

Table 9.2. A List of Representative Inorganic Constitutents of Living Tissues

Component	Formula
Sodium	Na^+
Potassium	K^+
Calcium	Ca^{++}
Magnesium	Mg^{++}
Water	H_2O
Compounds	NaCl, $CaCO_3$, PO_4^{3-}, SO_4^{2-}

Chemicals of the cellular pool are obtained from outside. Animal cells are bathed in an extracellular fluid having several chemi-

cals. However, the cells are able to maintain their specific cellular pool through selective intake of materials from outside, their metabolism inside the cell and selective elimination of specific chemicals. This is done through membranous covering of cell organelles and the plasma membrane. The membranes possess selective permeability and retentivity.

The chemicals of the cellular pool function as raw materials for carrying out various biochemical reactions of the cell organelles and the cell. Depending upon their **molecular weight** and **solubility*** they fall in two categories : micromolecules and macromolecules. **Micromolecules** or **biomicromolecules** are small sized simple chemicals that have low molecular weight (inbetween 18 to 800 daltons *i.e.*, less than 1000 daltons), higher solubility (if polar) and simple conformation. These are found in acid soluble pool. Micromolecules may be inorganic or organic and include water, gases, minerals, sugars, amino acids and nucleotides. **Macromolecules** or **biomacromolecules** are large sized complex chemicals that have high molecular weight, (usually 10000 daltons and above except lipids), low solubility and complex conformation. These are found in acid insoluble fraction. They usually produce colloidal complex. They are always organic. Macromolecules generally belong to four classes of organic compounds— carbohydrates, lipids, proteins and nucleic acids. Except lipids, other macromolecules are formed by polymerisation (condensation) of sub-units called **monomers**. They produce chains of variable lengths. Because of their formation from monomers by polymerisation, these chains are also called **polymers**.

(a) Proteins are polymers of different aminoacids. These amino acids are linked by **peptide bond** (CONH) formed by dehydration (polymerisation) between COOH group of one amino acid and NH_2 group of next amino acid with the removal of H_2O. (Fig. 9.1).

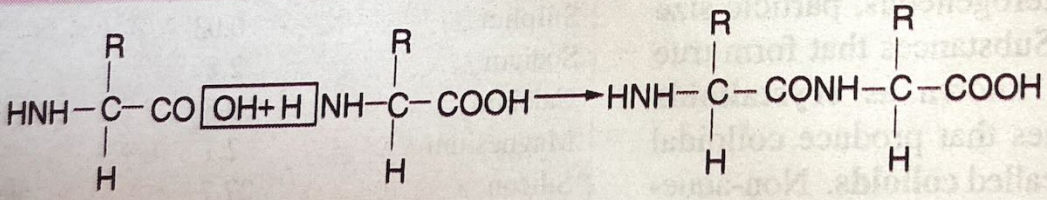

Fig. 9.1. Formation of a peptide bond between two amino acid.

(b) In nucleic acids, the phosphate molecule links 3′ C of sugar of one nucleoside to the 5′ C of sugar of next nucleoside, releasing 2 water molecules to form a 3′ –5′ phosphodiester bond (O—HPO_2—O ; Fig. 9.3).

(c) In polysaccharides, the monosaccharides are linked by glycosidic bond (C–O–C) formed by dehydration between two carbon atoms of two adjacent monosaccharides (Fig. 9.2).

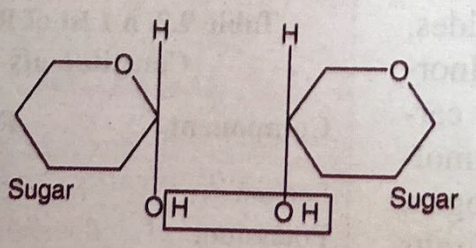

Fig. 9.2. Formation of a glycosidic bond.

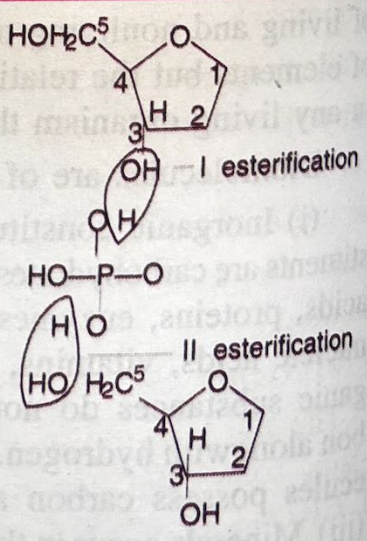

Fig. 9.3. Formation of a 3′ –5′ Phosphodiester bond.

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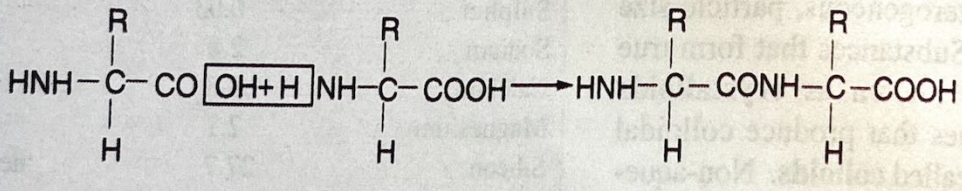

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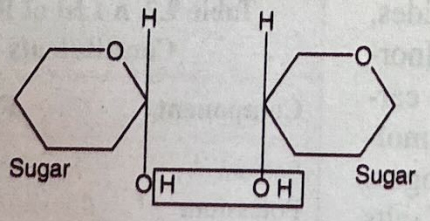

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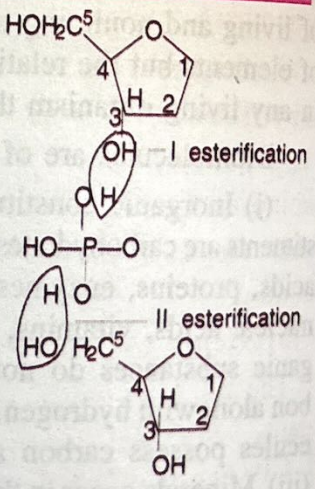

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Large sized macromolecules assume a three-dimensional shape which enables them to function as structural components, nutrient stores, store-house of genetic information, energy sources, molecular messengers, enzymes, etc.

Lipids are considered to be macromolecules, though they have molecular weight less than 800 daltons. Actually lipids are not water soluble. They get separated in acid insoluble fraction alongwith other macromolecules. Otherwise lipids are not strictly macromolecules.

The average composition of living cell is water 70–90%, proteins 10–15%; carbohydrates 3%; lipids 2%; Nucleic acids 5–7%, ions 1%.

CARBOHYDRATES

Carbohydrates are polyhydroxy aldoses, ketoses and their condensation products. Aldoses bear terminal aldehyde or —CHO group (*e.g.*, glyceraldehyde, xylose, ribose, glucose, galactose) while ketoses have an internal ketone or —CO— group (*e.g.*, dihydroxy acetone, erythrulose, ribulose, xylulose, fructose, sedoheptulose). The term carbohydrate is derived from french term *hydrate de carbone* indicating that it is a hydrate of carbon or $C_n(H_2O)_n$ or $(CH_2O)_n$. Accordingly carbohydrates are also defined as organic substances having carbon, hydrogen and oxygen where hydrogen and oxygen occur in the ratio of 2 : 1 as found in water (H_2O). Carbohydrates are directly produced during photosynthesis of autotrophic plants. They, therefore, constitute major part of the dry weight of plants (upto 80%).

Carbohydrates are also called **saccharides** (Gk. *sakcher–* sugar*)* because their basic components are sugars. Carbohydrates are of two types, small and complex. Small carbohydrates are soluble and have low molecular weight. Commonly they are sweet to taste and are called **sugars**. Small carbohydrates are further differentiated into monosaccharides, derived monosaccharides and oligosaccharides. Large carbohydrates are called polysaccharides.

Small Carbohydrates

1. Monosaccharides

They are those sugars or simple carbohydrate monomers which cannot be hydrolysed further into smaller components. They have a general formula of $C_nH_{2n}O_n$. Depending upon the number of carbon atoms, monosaccharides are of five types.

(a) **Trioses** (having 3 carbon atoms, $C_3H_6O_3$), *e.g.*, Glyceraldehyde, dihydroxyacetone.

(b) **Tetroses** (having four carbon atoms, $C_4H_8O_4$), *e.g.*, Erythrose, Threose.

(c) **Pentoses** (having five carbon atoms, $C_5H_{10}O_5$), *e.g.*, Ribose, Xylose, Deoxyribose, Arabinose, Ribulose. Deoxyribose is an exception because it has a formula of $C_5H_{10}O_4$.

(d) **Hexoses** (having six carbon atoms, $C_6H_{12}O_6$), *e.g.*, Glucose, Fructose, Galactose, Mannose.

(e) **Heptoses** (having seven carbon atoms, $C_7H_{14}O_7$), *e.g.*, Sedoheptulose, Glucoheptose.

The aldose or ketose group can also react with an alcoholic or nitrogen group of another organic compound. The bond thus formed is called **glycosidic bond** (C—O—C or C—N—C). Glycosidic bonds are also established during the condensation of monosaccharides for the formation of oligosaccharides and polysaccharides. A molecule of water is usually produced at each condensation. It is, therefore, an example of **dehydration synthesis**.

Pentoses and hexoses exist in both open chains as well as ring forms. There are two types of ring forms, **pyranose** and **furanose** (Fig. 9.4). Pyranose has hexagon structure with five carbons and one oxygen. Furanose has a pentagon structure with four carbons and one oxygen. Both pyranose and furanose types are further distinguished into α and β types.

In α-form the hydroxyl group nearer the oxygen atom of the ring is written below while in β–form it is written above. Many monosaccharides have asymmetric carbons and are able to rotate polarised light to right side (D, or dextrorotatory, +) or left side (L, or laevorotatory, –). Amongst sugars D-isomers are more common.

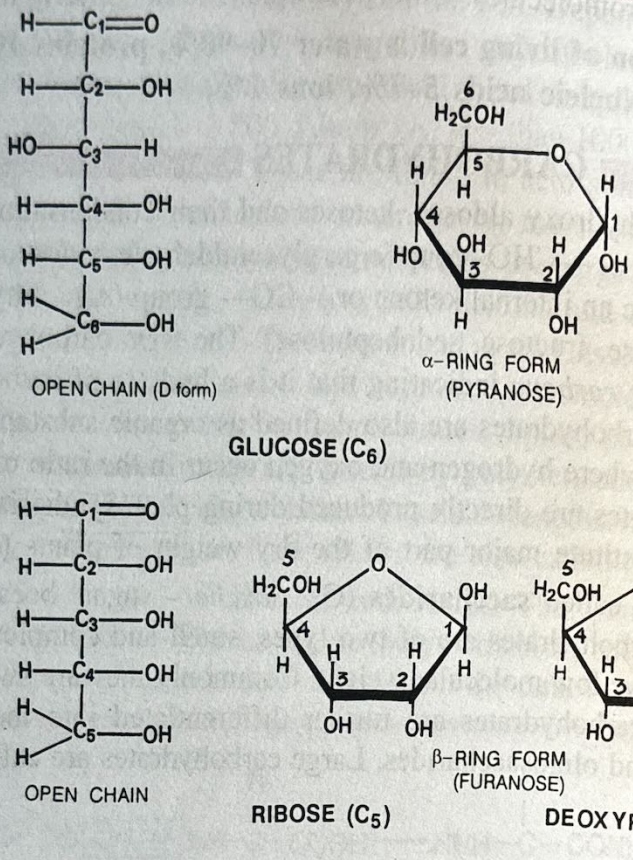


Fig. 9.4. Open chains and ring forms of a common hexose and two pentoses.

Hexoses are generally white crystalline and sweet substances. Fructose is the **sweetest** of all naturally occurring sugars. It is also called **fruit sugar** because of its common occurrence in fruits (exception Grape). Nectar and honey contain fructose. The same is called **laevulose** (=levulose) because of its laevorotatory nature. Glucose is **blood sugar** and common respiratory substrate which is also called **grape sugar**, corn sugar. It is called **dextrose** being dextrorotatory.

2. Derived Monosaccharides

Monosaccharides are modified variously to form a number of different substances. The important derivatives are : (i) **Deoxy Sugar**. Deoxygenation of ribose produces deoxyribose. The latter is a constituent of deoxyribotides found in DNA. (ii) **Amino Sugars**. The monosaccharides have an amino group (—NH_2). Glucosamine forms chitin, fungus cellulose, hyaluronic acid and chondroitin sulphate. Galactosamine is similarly a component of chondroitin sulphate. (iii) **Sugar Acid**. Ascorbic acid is a sugar acid. Glucuronic acid and galacturonic acid occur in mucopolysaccharides. (iv) **Sugar Alcohol**. Glycerol is involved in lipid synthesis. Mannitol is storage alcohol in some fruits and brown algae.

3. Oligosaccharides

They are small carbohydrates which are formed by condensation of 2–9 monosaccharides. Therefore, oligosaccharides belong to the category of **compound carbohydrates** (polysaccharides are also compound carbohydrates). Depending upon the number of monosac-

charide molecules condensed to form oligosaccharides, the latter are known as **disaccharides** (*e.g.*, sucrose, lactose), **trisaccharides** (*e.g.*, raffinose), **tetrasaccharides** (*e.g.*, stachyose), pentasaccharides, hexasaccharides, heptasaccharides, etc. Larger oligosaccharides having branched or unbranched chains occur attached to cell membranes.

The smallest and the commonest oligosaccharides are disaccharides. They are formed by the condensation of two monosaccharide molecules. Hexosan disaccharides are the best known, *e.g.*, sucrose, maltose, lactose, trehalose. **Trehalose** is found in some fungi and haemolymph of some insects. **Sucrose** is the commercial sugar which is also called cane sugar because most of it is obtained from Sugarcane (*Saccharum officinarum*). A large quantity is also obtained from Sugar Beet (*Beta vulgaris*). Sucrose is formed by condensation of one molecule each of glucose and fructose. Glycosidic bond is established between carbon atom 1 of glucose and carbon atom 2 of fructose. A molecule of water is released.

$$\underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Fructose}}{C_6H_{12}O_6} \longrightarrow \underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} + H_2O$$

The union is in the aldehyde region of glucose and ketone region of fructose. Unlike its components (glucose and fructose), sucrose is a **nonreducing sugar**. In sucrose, fructose occurs in the furanose (pentagon) form while glucose is in pyranose (hexagon) state (Fig. 9.5). Sucrose is the storage carbohydrate of Sugarcane and Sugarbeet.

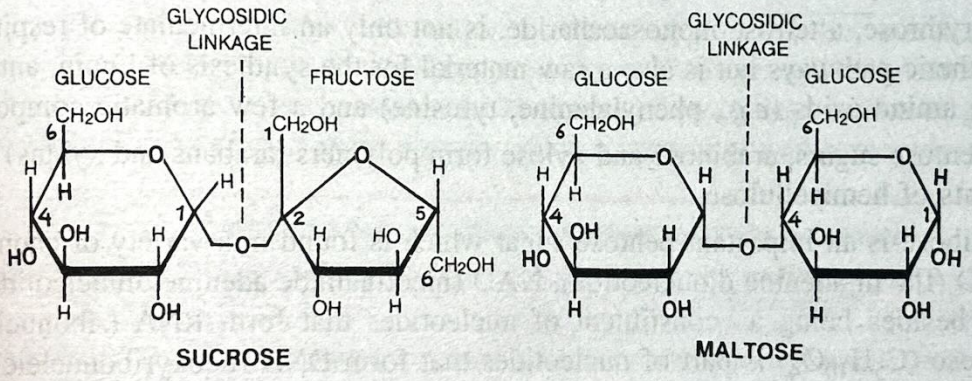


Fig. 9.5. Two common disaccharides.

Maltose or **malt sugar** is named so because of its occurrence in malted grains of Barley. It is also found in detectable amounts in most germinating seeds and tissues where starch is being broken down. It is a **reducing sugar** which is formed by condensation of two molecules of glucose (Fig. 9.5). Glycosidic bond (α 1 $\rightarrow$4) is established between carbon 1 of one glucose molecule and carbon 4 of second glucose molecule.

Lactose or **milk sugar** is named so because it is found naturally in milk. It is a reducing sugar which is formed inside mammary glands by β 1$\rightarrow$4 condensation of two hexose molecules, glucose and galactose. Souring of milk is due to conversion of lactose into lactic acid. Trisaccharide **raffinose** is also a reducing sugar. It is formed of glucose, fructose and galactose.

Sweetening index of sucrose, maltose and lactose is respectively 100, 32 and 16. It is 170 for fructose, 40000 for saccharin and 200,000 for monellin (a protein).

Reducing Sugars. They are those sugars which can reduce Cu^{2+} ions to Cu^{+} state. The property is found in all those saccharides which possess free aldose or ketose groups. All monosaccharides have this ability. Amongst disaccharides, sucrose is non-reducing because both aldose group of glucose and ketose group of fructose are lost due to formation of

glycosidic bond between the two. However, some other disaccharides (*e.g.*, lactose, maltose) possess the reducing groups. The reaction is also used in detecting glucose in urine. Two **types of tests** are used, **Fehling's solution** and **Benedict's solution**. The latter is more common. The solution contains blue coloured alkaline solution of copper (cupric) sulphate. Reducing sugar changes into insoluble reddish cuprous oxide on gentle heating. Cuprous oxide separates as precipitate. The final precipitate may appear green, yellowish, orange to brick red depending upon the amount of reducing sugar.

Differences between Reducing and Nonreducing Sugars

Reducing Sugars	*Nonreducing Sugars*
1. The sugars have a free aldehyde (—CHO) or ketonic (—CO) group.	1. A free aldehyde or ketonic group is absent.
2. The sugars can reduce cupric ions of Benedict's or Fehling's solution to cuprous ions.	2. They do not reduce cupric ions of Benedict's or Fehling's solution to cuprous ions.

Functions of Small Carbohydrates

1. Trioses, glyceraldehyde and dihydroxy acetone, are important intermediates of both respiratory and photosynthetic pathways.

2. Erythrose, a tetrose monosaccharide, is not only an intermediate of respiratory and photosynthetic pathways but is also a raw material for the synthesis of lignin, anthocyanins and some amino acids (*e.g.*, phenylalanine, tyrosine) and a few aromatic compounds.

3. Pentose sugars, arabinose and xylose form polymers (arabans and xylans) which are constituents of hemicellulose.

4. Ribose is an important pentose sugar which is found in a variety of chemicals like CoA, FAD (flavin adenine dinucleotide), NAD (nicotinamide adenine dinucleotide), NADP and ATP besides being a constituent of nucleotides that form RNA (ribonucleic acid). Deoxyribose ($C_5H_{10}O_4$) is part of nucleotides that form DNA (deoxyribonucleic acid).

5. Ribulose 1,5 biphosphate or RuBP (a phosphorylated pentosan) is the acceptor of carbon dioxide in photosynthesis.

6. Glucose is the main respiratory substrate. Fructose can also function similarly.

7. Fats and amino acids are formed from glucose and other sugars.

8. Oligosaccharides attached to cell membranes form **cell coat** or **glycocalyx**. They are important for (a) cell **recognition** (b) cell **attachment** (c) **receptor** molecules (for receiving and responding to external stimuli) (d) **antigen** specificity for human blood groups (A, B, Rh) (e) components of **antibodies** which are large molecules with attached carbohydrates (f) **glycoproteins** of some viral coats for attaching to and invading host cells.

9. Some plants store oligosaccharides as reserve food, *e.g.*, sucrose (sugarcane and sugarbeet).

10. Monosaccharides are polymerised to form structural carbohydrates (*e.g.*, cellulose, ligno-cellulose) and storage carbohydrates, starch in plants and glycogen in animals.

POLYSACCHARIDES

They are complex carbohydrates which are formed by polymerisation of large number of monosaccharide monomers (more than 10 but usually numerous). Polysaccharides are also called **glycans**. They are long chained. The right end of a polysaccharide is **reducing**

end while the left end is named as **nonreducing end**. The chains may be branched or unbranched. Depending upon the composition, polysaccharides are of two types, homopolysaccharides and heteropolysaccharides.

Homopolysaccharides or homoglycans are those complex carbohydrates which are formed by polymerisation of only one type of monosaccharide monomers. For example, starch, glycogen and cellulose are composed of a single type of monosaccharide called glucose. Depending upon monosaccharide unit involved, polysaccharide is called **glucan** (made of glucose), **fructan** (made of fructose), **xylan** (having xylose), **araban** (made of arabinose), **galactan** (formed of galactose), etc.

Heteropolysaccharides or heteroglycans are those complex carbohydrates which are produced by condensation of two or more types of monosaccharides or their derivatives, *e.g.*, agar, chitin peptidoglycan, arabanogalactans, arabanoxylans, etc.

During the formation of polysaccharides a molecule of water is released at each condensation.

$$\underset{\text{Monosaccharide}}{n\,(C_6H_{12}O_6)} \longrightarrow \underset{\text{Polysaccharide}}{(C_6H_{10}O_5)_n} + nH_2O$$

This reduces the bulk, makes the polysaccharide almost insoluble and decreases its effect on water potential or osmotic potential of the cell. They are, therefore, **ideal for storage** and as structural components.

Unlike sugars, polysaccharides are not sweet. Being large sized, they are unable to pass through cell membranes though their components do so. Passage is allowed through active process. Another method of their passage is by means of secretion vesicles of golgi apparatus. Polysaccharides are of 3 main types— storage, structural and mucopolysaccharides.

Differences between Oligosaccharides and Polysaccharides

Oligosaccharides	*Polysaccharides*
1. They contain 2 – 9 monosaccharide residues.	1. Polysaccharides possess a large number of monosaccharide residues.
2. Oligosaccharides are soluble in water.	2. Polysaccharides are usually insoluble.
3. They are commonly sweet to taste.	3. Sweetness is absent.
4. Transport of carbohydrates occurs in oligosaccharide state.	4. Polysaccharides are not involved in transport.
5. Storage in oligosaccharide state is less common.	5. Storage occurs in polysaccharide state.
6. They form component of external surface of plasma membrane and cell coat. **Examples.** Sucrose, Maltose, Raffinose.	6. They form component of cell wall. **Examples.** Starch, Glycogen, Cellulose.

A. Food Storage Polysaccharides

They are those polysaccharides which serve as reserve food. At the time of need, storage polysaccharides are hydrolysed. Sugars thus released become available to the living cells for production of energy and biosynthetic activity. There are two main storage polysaccharides— starch and glycogen.

1. **Starch.** It is the storage polysaccharide of most plants. Human beings obtain it from cereal grains (*e.g.*, rice, wheat), legumes (pea, gram, beans), potato, tapioca, banana etc. It is polyglucan homosaccharide and is formed as an end product of photosynthesis. Starch

is stored either inside chloroplasts or special leucoplasts called amyloplasts. Starch occurs in the form of microscopic granules called **starch grains**. Starch grains may occur singly or in groups. The two types are known as simple and compound starch grains. Starch grains may be rounded, oval, polygonal or rod shaped in outline (Fig. 9.6). Each grain has a number of shells or layers arranged in concentric or eccentric fashion around a proteinaceous point called **hilum**.

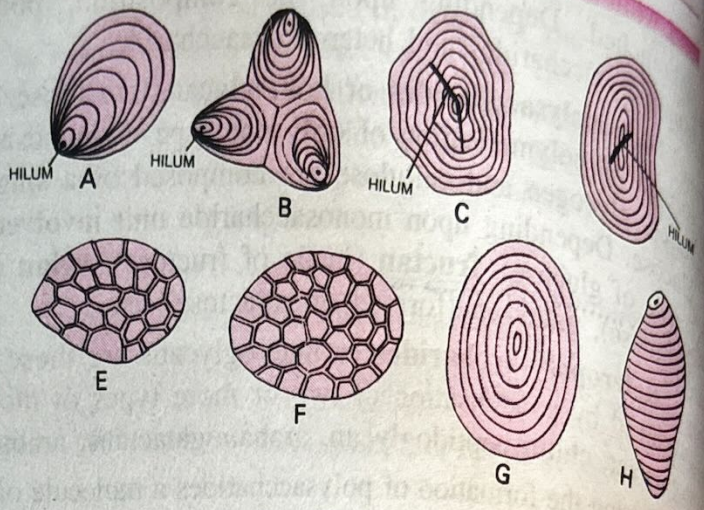


Fig. 9.6. Types of starch grains. A, simple starch grain of Potato. B, compound starch grain of Potato. C, a simple starch grain of Maize. D, a simple starch grain of Pea. E, a compound starch grain of Rice. F, a compound starch grain of Oat. G, a simple starch grain of Wheat. H, a simple starch grain of Banana.

Starch consists of two components, amylose and amylopectin (Fig. 9.7). Amylose is more soluble in water than amylopectin. In general, 20–30% of starch consists of amylose and the rest as amylopectin. Waxy starch of some varieties of Maize and other cereals consists entirely of amylopectin. On the other hand, the starch of some varieties of Pea having wrinkled surface may have as much as 98% of amylose.

Both amylose and amylopectin are formed by the condensation of α -D-glucose (pyranose forms). Amylose is in the form of a continuous straight but helically arranged chain where each turn contains about six glucose units. The successive glucose units are linked together by 1–4 α-linkages, that is, the link is between carbon atom 1 of one and carbon atom 4 of the other (Fig. 9.8). A molecule of water is lost during the formation of the linkage. A straight chain of amylose consists of 200–1000 glucose units.

Amylopectin contains a large number of glucose units (2000–200,000). Besides a straight chain it bears several side chains which may be branched further. Branching is usually at intervals of about 25 residues. At the place of origin of a side chain, the carbon atom 6 of a glucose residue of a straight chain is linked to the carbon atom 1 of the first glucose unit of side chain (1–6 α-linkage). Wolform and Thompson (1956) have also reported 1→3 linkages in case of amylopectin.

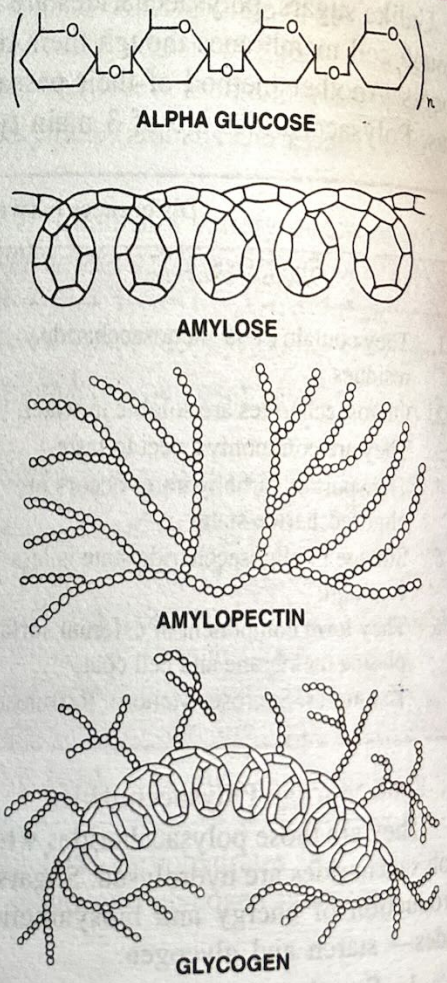


Fig. 9.7. Diagrammatic structure of storage polysaccharide.

Test. Amylose fraction gives blue-black colour with iodine solution (Iodine-Potassium iodide solution) while amylopectin fraction gives red-violet colour.

2. **Glycogen**. It is the polysaccharide food reserve of animals, bacteria and fungi. Glycogen is popularly called **animal starch**. Glycogen is mainly stored inside liver (upto 0.1 kg) and muscles. In shape the complex carbohydrate appears as ellipsoid flattened granules that lie freely inside the cells. The polysaccharide gives reddish colour with iodine. Chemically, it is similar to starch. It has about 30,000 glucose residues and a molecular weight of about 4.8 million. Glucose residues are arranged in a highly branched bush like chains. There are two types of linkages 1–4 α -linkages in the straight part and 1–6 linkages in the area of branching. The straight part is helically twisted with each turn having six glucose units. The distance between two branching points is 10–14 glucose residues (Fig. 9.7).

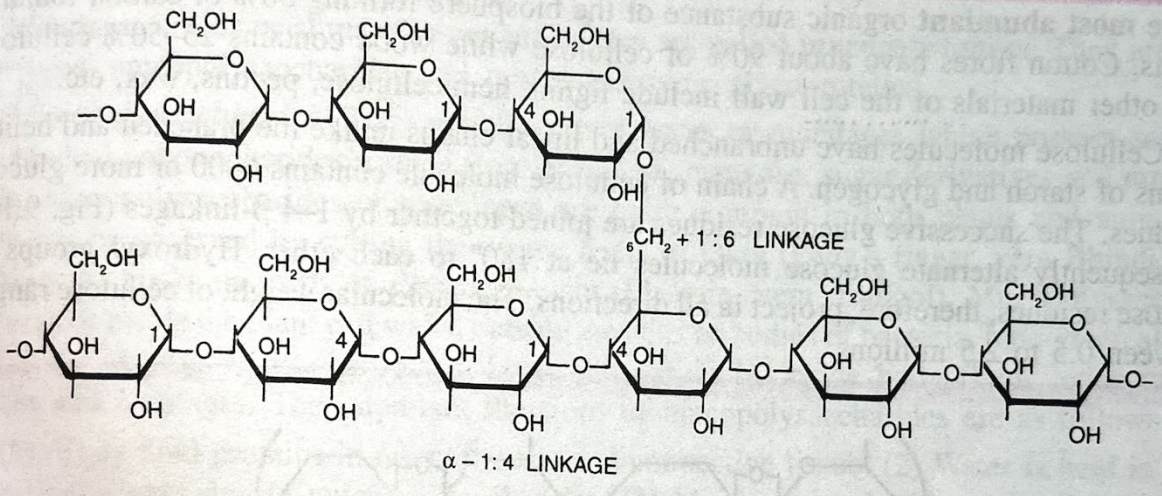


Fig. 9.8. Structure of starch.

3. **Inulin**. It is a **fructan** storage polysaccharide of roots and tubers of *Dahlia* and related plants. Inulin is not metabolised in human body and is readily filtered through the kidney. It is, therefore, used in testing of kidney function, especially glomerular filtration.

B. Structural Polysaccharides

They are polysaccharides that take part in forming the structural frame work of the cell walls in plants and skeleton of animals. Structural polysaccharides are of two main types : chitin and cellulose.

1. **Chitin**. It is the **second most abundant** organic substance. Chitin is a complex carbohydrate of heteropolysaccharide* type which is found as the structural component of fungal walls and exoskeleton of arthropods. In fungal walls, chitin is often known as fungus cellulose. Chitin is soft and leathery. Therefore, it provides both strength and elasticity. It becomes hard when impregnated with certain proteins and calcium carbonate.

In chitin, basic unit is not glucose but a nitrogen containing glucose derivative known as N-acetyl glucosamine. Chitin has an unbranched configuration. Monomers are joined together by 1– 4 β-linkages (Fig. 9.9). Adjacent residues lie at 180°. Molecules occur in parallel and are held together by hydrogen bonds.

* Some consider chitin to be **homopolymer**.

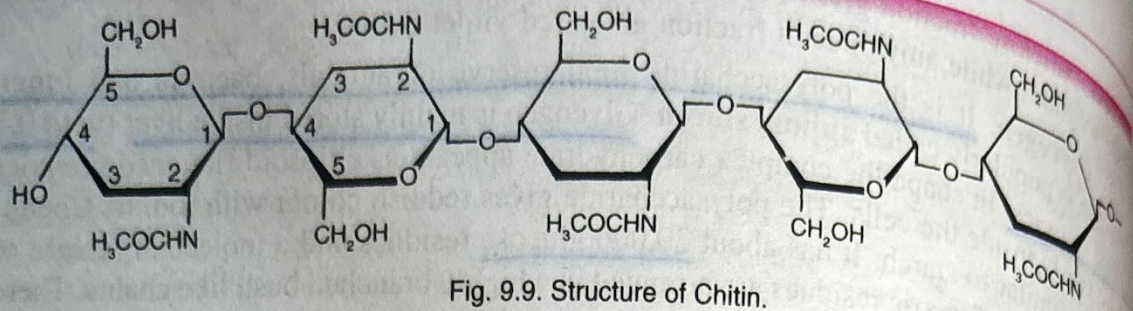


Fig. 9.9. Structure of Chitin.

2. **Cellulose**. It is fibrous homopolysaccharide of high tensile strength which forms a structural element of cell wall in all plants, some fungi and protists. **Tunicin** of tunicates (=ascidians) is related to cellulose (also called animal cellulose). In absolute terms, cellulose is the **most abundant** organic substance of the biosphere forming 50% of carbon found in plants. Cotton fibres have about 90% of cellulose while wood contains 25–50% cellulose. The other materials of the cell wall include lignin, hemicellulose, pectins, wax, etc.

Cellulose molecules have unbranched and linear chains unlike the branched and helical chains of starch and glycogen. A chain of cellulose molecule contains 6000 or more glucose residues. The successive glucose residues are joined together by 1–4 β-linkages (Fig. 9.10). Consequently alternate glucose molecules lie at 180° to each other. Hydroxyl groups of glucose residues, therefore, project in all directions. The molecular weight of cellulose ranges between 0.5 to 2.5 millions.

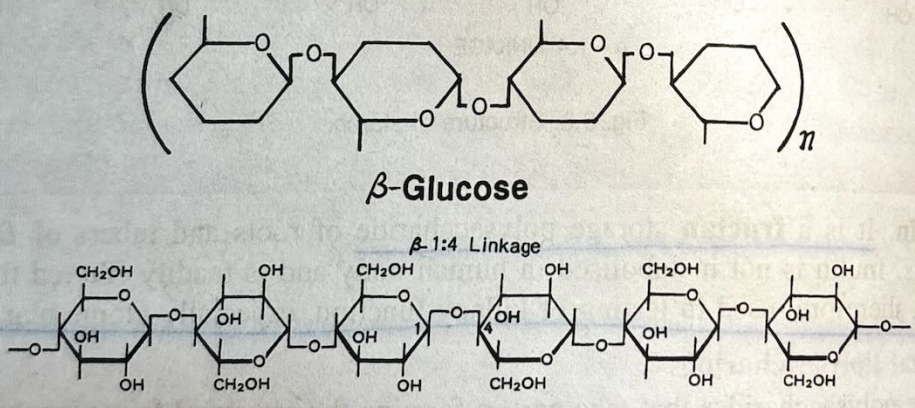


Fig. 9.10. Structure of cellulose.

Cellulose molecules do not occur singly. Instead a number of chains are arranged in close antiparallel fashion. The molecules are held together by intermolecular hydrogen bonds between hydroxyl group at position 6 of glucose residues of one molecule and glycosidic oxygen between two glucose residues of the adjacent molecule. There is also intramolecular strengthening of the chain by the formation of hydrogen bonds between hydroxyl group at position three and oxygen atom of the next residue. About 2000 cellulose chains or molecules are packed together to form a microfibril visible under the electron microscope.

Importance. (1) Cellulose constitutes the bulk of human food. However, due to being polymer of β-glucose, cellulose is not acted upon by amylases present in human digestive juices. In humans, cellulose has a **roughage** value which keeps the digestive tract in functional fitness. (2) Cellulose is an important constituent of diet for ruminants like cows and buffaloes. The stomach of ruminants contain micro-organisms capable of digesting or

breaking down cellulose. Termites and snails also possess micro-organisms in their gut for this purpose. (3) Microbes are used in producing soluble sugars from cellulose. The sugars are then allowed to undergo fermentation for obtaining ethanol, butanol, acetone, methane, etc. (4) Cellulose rich wood is employed in building furniture, tools, sports articles, paper etc. (5) Depending upon the percentage of cellulose present in the fibres, the latter are used in textiles (*e.g.*, Cotton, Linen), preparation of sacs (*e.g.*, Jute) or ropes (*e.g.*, Hemp, China Jute, Deccan Hemp). (6) Rayon and cellophane are formed of cellulose xanthate. (7) Cellulose acetate is obtained by treating wood pulp with acetic acid, acetic anhydride and a catalyst. Cellulose acetates are used in preparing fibres for double knits, tericot, wrinkle proof, and moth proof clothing. Cigarette filters are also prepared from these fibres. Other uses of cellulose acetates include preparation of plastics and shatter proof glass. (8) Cellulose nitrate is used in propellent explosives. (9) Carboxymethyl cellulose is used as emulsifier and smoothening reagent of ice creams, cosmetics and medicines.

C. Mucosubstances

Mucilage, mucus or slime forming substances are called **mucosubstances**. They are of two types, mucopolysaccharides and mucoproteins (= glycoproteins).

Mucopolysaccharides. They are slimy substances or mucilages which possess acidic or aminated polysaccharides formed from galactose, mannose, sugar derivatives and uronic acids. Mucopolysaccharides or **mucilages** are quite common in both plants and animals. They can be observed by cutting the unripe fruits of Okra (Lady's finger, vern Bhindi) or soaking the husks or seeds of *Plantago ovata* (Plantain, vern. Isabgol). Mucopolysaccharides occur inside the plant cell walls, outside the cells or bodies of bacteria, blue-green algae and many aquatic plants, cementing layer between cells, inside body fluids, connective tissues and cartilages. The important functions of mucopolysaccharides are as follows :

(1) They bind proteins in the cell walls and connective tissue. (2) Water is held in the interstitial spaces due to mucopolysaccharides. (3) Mucopolysaccharides occur in the cell walls of bacteria and blue-green algae. They form a layer of mucilage around them as well as other aquatic plants and protects the organisms against rotting effect of water, prevents desiccation, growth of epiphytes and attack of pathogens. (4) They provide lubrication in ligaments and tendons. (5) As keratan sulphate* (acetylglucosamine + galactose + sulphuric acid) they occur inside skin and cornea providing both strength and flexibility. (6) Chondroitin sulphate* (glucuronic or iduronic acid + acetyl aminogalactose) is the mucopolysaccharide found in the matrix of cartilage and connective tissue for support and elasticity. (7) Hyaluronic acid* (glucuronic acid +acetyl glucosamine) is the mucopolysaccharide met in extracellular fluid of animal tissues, vitreous humor of eye, synovial fluid, cerebrospinal fluid, etc. It also occurs in cementing material between animal cells as well as inside cell coat. (8) Husk of *Plantago ovata* contains mucilage which is used medicinally in treating intestinal problems. It relieves irritation. Mucilage present in *Aloe barbadensis* reduces inflammation. It is also purgative. (9) Mucopolysaccharide heparin (α-1,4 glucosamine + glucuronic acid) is blood anticoagulant. (10) **Agar.** Marine brown and red algae, called sea weeds, yield mucopolysaccharides of commercial value, *e.g.*, agar, alginic acid, carragenin, etc. **Agar** (agar-agar) is polymer of D-galactose, 3–6 anhydro L-galactose having sulphate esterification after every tenth galactose unit. It is used as culture medium in the laboratory, as laxative, stiffening, stabilising and emulsifying agent. It is obtained from cell wall of some red alae like *Gracilaria, Gelidium* and *Gelidiella*.

*They are proteoglycans where mucopolysaccharides are hybridised with small quantity of protein.

Pectin (Pectic Compounds). It is an acidic polysaccharide that occurs in the matrix of cell wall and middle lamella (as calcium pectate). Pectin is soluble in water and can undergo sol ⇌ gel interchange. Pectin is formed of galacturonic acid, galactose, methylated galacturonic acid and arabinose. It is used in making Jelly and Jams.

Hemicellulose. It is a mixture of polysaccharides of xylans, mannans, galactans, arabinogalactans and gluco-mannans. Hemicellulose occurs in the cell wall where it forms a link between pectic compounds and cellulose microfibrils.

Peptidoglycan (Murein, Mucopeptide). It is formed of heteropolysaccharide chains are cross-linked by short peptides (generally tetrapeptides). Heteropolysaccharide chains are formed of two alternate amino-sugar molecules, N-acetyl glucosamine (NAG) and N-acetyl muramic acid (NAM).

Lipopolysaccharide. It is a complex formed of lipid and polysaccharide which forms the outer membrane of Gram –ve bacteria. There is a glycolipid responsible for endotoxic activity, a core polysaccharide and an antigen specific variable chain. Lipopolysaccharide induces fever, shock and other toxic effects.

Mucoproteins (glycoproteins). The protein with conjugated mucosaccharides form mucus. These are found in stomach, nasal secretion, intestine, vagina and are antibacterial and protective in function.

Functions of Polysaccharides

1. Starch and glycogen are the major storage foods of organic world.
2. On hydrolysis storage carbohydrates provide both energy and carbon chains.
3. Chitin is the structural carbohydrate of fungal walls and exoskeleton of arthropods.
4. Cellulose is the structural substance of cell walls in most of the plants.
5. Cellulose is economically important in the production of furniture, shelter, fuel, paper, textiles, ropes, rayon, cellophane, plastics, shatter proof glass, propellent explosives, emulsifier and raw material for several fermentation products.
6. For human beings cellulose has a roughage value but it is a food for ruminants, snails and termites.
7. Mucilage present as a protective coating around aquatic plants, bacteria, blue-green algae and some animals is derived from polysaccharides.
8. Mucopolysaccharides in human body have several functions— lubrication of ligaments and tendons, formation of some types of body fluids like cerebrospinal fluid, synovial fluid, vitreous humor, providing strength and flexibility to skin, connective tissue and cartilage, binding of proteins in cell walls and holding water in interstitial spaces.
9. Heparin prevents blood clotting inside blood vessels of animals.
10. Some mucopolysaccharides have medicinal and other commercial importance, *e.g.*, husk of *Plantago ovata*, mucilage of *Aloe*, and alginic acid, agar, carragenin of marine algae.
11. Pectins form matrix of cell wall. As calcium pectate they produce middle lamella or cementing layer between adjacent plant cells. Pectins are commercial jellying agents.

LIPIDS

Lipids (Gk. *lipos*– fat; Bloor, 1943) are fatty acid esters of alcohols and related substances which are insoluble in water but get dissolved in a number of nonpolar organic solvents like ether, benzene, chloroform, acetone, etc. They can, however, form colloidal

complex and get dispersed uniformly in water in the form of minute droplets. The complex is called **emulsion**. Lipids are basically made of carbon, hydrogen and sometimes oxygen in which the content of oxygen is always small as compared to hydrogen and carbon. Many lipids contain small amounts of additional elements like phosphorus, nitrogen and sulphur. Common examples of lipids include butter, ghee, cooking oil, natural rubber, cholesterol, carotene of carrot or lycopene of tomato, eucalyptus oil, menthol, vitamins A, E and K. Depending upon their composition and characteristics, lipids are often classified into fats, cutin, suberin, waxes, phospholipids, glycolipids, carotenes and sterols. The basic component of all lipids are fatty acids.

Fatty Acids. Fatty acids are organic acids having hydrocarbon chains that end in a carboxylic group (—COOH). Hydrocarbon chains may be straight or have ring structure. Some fatty acids also possess hydroxyl groups. Length is 4–24 carbons. The common fatty acids have 16 or 18 carbons. Plants have the biosynthetic machinery to manufacture all types of fatty acids. Animals also have this machinery but many animals lack the mechanism to synthesise three fatty acids— linoleic, linolenic and arachidonic acids. They are called **essential fatty acids** (EFA; Burr and Burr, 1930). They must be present in the animal diet. Essential fatty acids occur in most edible oils like those of Sunflower, Groundnut, Cottonseed, Coconut, etc. Their deficiency produces **phrenoderma** or **follicular hyperkeratosis.**

Fatty acids are of two main types— **saturated and unsaturated**. Saturated fatty acids do not possess double bonds in their carbon chains. They have a general formula of $C_nH_{2n}O_2$, *e.g.*, $C_{16}H_{32}O_2$ (palmitic acid), $C_{18}H_{36}O_2$ (stearic acid).

$CH_3(CH_2)_{14}COOH$ — Palmitic acid

$CH_3(CH_2)_{16}COOH$ — Stearic acid

Unsaturated fatty acids possess one or more double bonds in their carbon chains. The general formula is $C_nH_{2n-2x}O_2$, *e.g.*, oleic acid (with one double bond, monoene, $C_{18}H_{34}O_2$), linoleic acid (with two double bonds, diene, $C_{18}H_{32}O_2$), linolenic acid (with three double bonds, triene, $C_{18}H_{30}O_2$), arachidonic acid (with four double bonds, tetraene, $C_{20}H_{32}O_2$).

$CH_3\ (CH_2)_7\ CH = CH\ (CH_2)_7\ COOH$

Oleic acid

$CH_3\ (CH_2)_4CH = CH\ CH_2\ CH = CH\ (CH_2)_7\ COOH$

Linoleic acid

$CH_3\ CH_2\ CH = CH\ CH_2\ CH = CH\ CH_2\ CH = CH\ (CH_2)_7\ COOH$

Linolenic acid

$CH_3\ (CH_2)_4CH = CHCH_2CH = CHCH_2CH = CHCH_2CH = CH(CH_2)_3COOH$

Arachidonic acid

Unsaturated fatty acids have lower melting point than the saturated fatty acids. Most plant lipids have unsaturated fatty acids while most animal lipids have saturated fatty acids. Aquatic animals of colder waters, however, possess unsaturated fatty acids. Carboxylic group of a fatty acid is **hydrophilic, polar** or water soluble while the rest of the hydrocarbon chain is **nonpolar, lipophilic, hydrophobic** or water insoluble. Polar end sticks to water so that fatty acids form a monomolecular layer over it. The water soluble end of fatty acid also reduces surface tension and increases the wetting or cleaning power of water. Soap action is actually the action of fatty acids contained in it.

Differences between Saturated and Unsaturated Fats

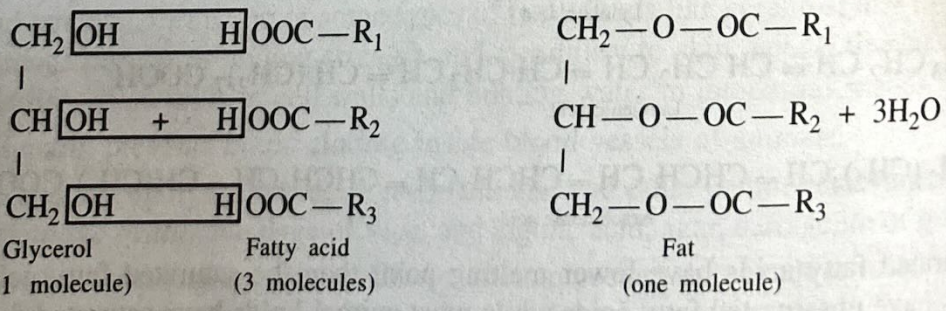
Saturated Fats	*Unsaturated Fats*
1. They do not possess any double bonds in their fatty acids.	1. They contain one or more double bonds in their fatty acids.
2. All carbon atoms are fully saturated.	2. Carbon atoms are unsaturated in the region of double bonds.
3. They have straight chains.	3. The chain bends at the double bond.
4. They are solid at ordinary temperature.	4. They are liquid at ordinary temperature.
5. They have higher melting point..	5. They have lower melting point.
6. Animal fats are mostly saturated fats.	6. Plant fats are generally unsaturated fats.
7. They increase blood cholesterol.	7. Unsaturated fats lower blood cholesterol.
8. Hydrogenation has no effect on saturated fats.	8. Hydrogenation converts unsaturated fats into saturated ones.
9. Essential fatty acids are absent.	9. Essential fatty acids are present.
10. They are not much affected by exposure to air.	10. On exposure to air, they tend to solidify.

Classification. Lipids are distinguishable into three types— simple, compound and derived. **Simple lipids** are formed from fatty acids and alcohol. They do not have any additional group, *e.g.*, fats, suberin, cutin, wax. **Compound** or **conjugated lipids** possess additional groups besides fatty acids and alcohol *e.g.*, phospholipids, glycolipids, lipoproteins (lipids linked to proteins). Derived lipids are either lipid-like chemicals (*e.g.*, sterols) or derivatives of lipids, *e.g.*, terpenes, prostaglandins.

1. Neutral or True Fats

They are **triglycerides** which are formed by esterification of three molecules of fatty acids with one molecule of trihydric alcohol, glycerol (glycerine or trihydroxy propane). Three molecules of water are eliminated. The word triglyceride refers to the number of three molecules of fatty acids esterified to a molecule of glycerol. If the number of fatty acids attached to a glycerol happens to be two, the ester is called diglyceride, or monoglyceride if there is only one molecule of fatty acid attached to a glycerol molecule.

In fats the three fatty acids are only rarely similar (*e.g.*, tripalmitin, tristearin, triolein). They are called **pure fats**. Usually they are dissimilar or two of the three fatty acids are similar. They are known as **mixed fats**, *e.g.*, Butter. Fats are named after the names of fatty acids, *e.g.*, dipalmito-stearin, palmito-oleio-stearin, steario-oleio-palmitin.



Most neutral fats are mixtures of different triglycerides. For commercial use fats are differentiated into **hard fats** and **oils**. Oils are those fats which are liquid at room temperature of 20°C. This is because they have low melting point, *e.g.*, groundnut (peanut) oil, cotton seed oil, rape seed oil, mustard oil, sesame oil, sunflower oil, safflower oil, etc. The low melting property is actually the property of fatty acids. Oils are richer in either unsaturated fatty acids or fatty acids having small carbon chains. Unsaturated fatty acids can combine

with oxygen and other chemicals. Therefore, exposed oils have a tendency to solidify. Hence, they are called **drying oils**. Edible oils can be converted into hard fats through the process of **hydrogenation**. In hydrogenation unsaturated fatty acids are changed to saturated state. Vanaspati or vegetable ghee and margarine (emulsified animal or plant fat) are obtained from oils through hydrogenation.

Oils with polyunsaturated fatty acids (fatty acids with more than one double bond) are called **polyunsaturates (PUFA)**. They are recommended by physicians to persons having hypertension, high blood cholesterol and other cardiovascular diseases. It is because poly-unsaturated fatty acids lower blood cholesterol. Edible oils having polyunsaturated fatty acids are safflower and sunflower oils.

Hard fats are solid at room temperature of 20°C. They contain long chain saturated fatty acids, *e.g.*, animal fat. Butter is soft because it contains good quantity of short chain fatty acids.

2. Waxes

They are fatty acid esters of long chain monohydric alcohols like cytyl, ceryl or mericyl. Other fat like substances also occur in waxes.

Plant waxes occur in cuticle (along with cutin) and as greyish waxy coating or bloom around the plant organs. Wax found on the upper surface of floating leaves prevents wetting and submergence. Wax found on the surface of land plants is useful in reducing transpiration.

In animals cutaneous glands are known to secrete wax **lanolin** for forming a protective water insoluble coating on animal fur. **Cerumen** or ear wax is secreted by cutaneous glands for lubricating ear drum. Bees build their hives from wax secreted by their abdominal glands. **Beeswax** is a complex of several waxes. The major component is ester of palmitic acid ($C_{16}H_{32}O_2$) and mericyl alcohol (= triacontanol, $C_{30}H_{61}OH$). Second major component is ester of palmitic acid with hexacosonal ($C_{26}H_{53}OH$).

$$C_{15}H_{31}COOH + C_{30}H_{61}OH \longrightarrow C_{15}H_{31}.CO.O.C_{30}H_{61}$$
triacontanyl palmitate

$$C_{15}H_{31}COOH + C_{26}H_{53}OH \longrightarrow C_{15}H_{31}.CO.O.C_{26}H_{53}$$
hexacosyl palmitate

Tuberculosis and leprosy bacteria produce a wax called **wax-D** which is a major factor in their pathogenicity.

Paraffin wax is obtained from petroleum. Candles are made of paraffin wax and stearic acid. Waxes are used in cosmetics and polishes. Waxed paper is used for wrapping.

3. Cutin

It is a complex lipid produced by cross-esterification and polymerisation of hydroxy fatty acids, as well as other fatty acids with or without esterification by alcohols other than glycerol. Cutin occurs in the aerial epidermal cell walls as well as a separate layer of cuticle on the outside of these epidermal cells. Cuticle has 50–90% cutin.

Cutin reduces the rate of transpiration. It also binds epidermal cells.

4. Suberin

It is a mixture of fatty material having condensation products of glycerol and phellonic acid or its derivatives. Suberin makes the cell wall strong and impermeable. It occurs in the walls of cork cells and endodermal cells.

5. Phospholipids (Common Membrane Lipids)

They are triglyceride lipids where one fatty acid is replaced by phosphoric acid which is often linked to additional nitrogenous groups like choline (in lecithin), ethanolamine (in cephalin), serine or inositol. Phospholipids are **amphipathic** carrying both hydrophilic polar and hydrophobic nonpolar groups.

The hydrocarbon chains of the two fatty acids function as **hydrophobic non-polar tails** of the phospholipid molecule. The phosphate and additional group (nitrogenous or non-nitrogenous) behave as **hydrophilic polar head** group of the molecule (Fig. 9.11). In aqueous medium the phospholipid molecules arrange themselves to form a double layer or bilayer (Fig. 9.12). The polar or hydrophilic heads of molecules form the two surfaces which are in contact with water. The hydrophobic or nonpolar tails of the phospholipid molecules are towards the centre of the bilayer. Lipid bilayer is the basic component of all cell membranes.

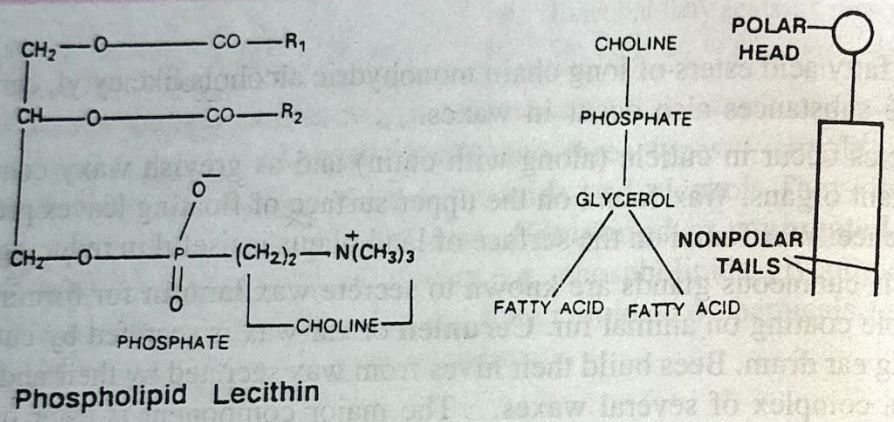


Fig. 9.11. Phospholipid lecithin showing polar head group made of phosphate as well as choline and two nonpolar fatty acid tails.

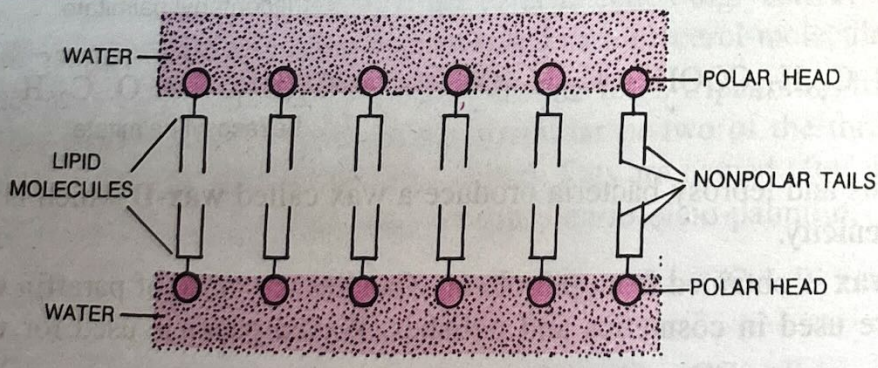


Fig. 9.12. Lipid bilayer formed in aqueous medium.

6. Sphingolipids

They are lipids having amino alcohol sphingosine. **Sphingomyelins** contain an additional phosphate attached to choline like phospholipids. They occur in myelin sheath of nerves. **Cerebrosides** possess sugar residue galactose. They occur in nerve membranes. **Gangliosides** possess sugar residues glucose, galactose, sialic acid and acetyl glucosamine. They influence ion transport through the membrane as well as function as receptors of viral particles. Gangliosides occur in grey matter. Excessive accumulation of gangliosides produces disorders like Tay-Sachs disease.

Since cerebrosides and gangliosides contain sugar residues, they are also called **glycolipids.**

7. Lipoproteins

Lipoproteins are composed of lipids and proteins. They are present in blood, milk and egg yolk.

8. Terpenes

They are lipid like hydrocarbons formed of isoprene (C_5H_8) units. Steroids like cholesterol are also derived from terpenes having 6 isoprene units. Essential oils of plant origin are terpenes with 2–4 isoprene units, *e.g.*, camphor, menthol. Gibberellins are a group of plant growth hormones with four isoprene units. An equal number of isoprene units occur in vitamin A, E and K. Phytol or tail of chlorophyll *a* molecule is a terpene with 4 isoprene units. Carotenoids (carotenes and xanthophylls) have 8 isoprene units. Natural and synthetic rubbers are terpenes with thousands of isoprene units arranged in linear fashion.

9. Prostaglandins

They are derivatives of arachidonic acid and other 20 Carbon fatty acids which have several functions like vasodilation, vasoconstriction, bronchoconstriction, acid production in stomach, cell communication and hormone modulation.

10. Steroids

They are a group of complex lipids that possess a hydrogenated cyclopentano-perhydrophenanthrene ring system (Fig. 9.13). Position of carbon atoms are numbered. Attached to carbon atoms 10 and 13 are present methyl groups (having carbon atoms 19 and 18 respectively). In cholesterol a side chain is attached to carbon 17. Sterols have one or more hydroxyl groups. Others have carbonyl or carboxyl groups. Major groups of steroids are :

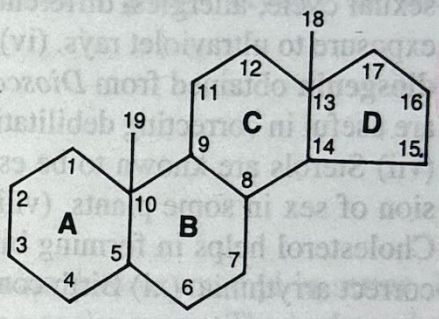


Fig. 9.13. Cyclopentano-hydrophenanthrene ring system.

1. **Sterols.** Steroids with 8–10 carbon long aliphatic side chain at carbon 17 and at least one alcoholic hydroxyl (*e.g.*, at position 3), *e.g.*, cholesterol, ergasterol, coprosterol, stigmasterol, sitosterol, campesterol.

2. **Bile Acids.** They are steroid carboxylic acids derived from cholesterol, *e.g.*, glycocholic acid, taurocholic acid.

3. **Gonadal (Sex) Hormones.** Estradiol (oestrogen), testosterone, progesterone.

4. **Adrenocortical Hormones.** Corticosterone.

5. **Cardiac Glycosides.** They contain carbohydrate steroid complex with steroid nucleus having unsaturated lactone ring attached to carbon 17 *e.g.*, digitoxin (from *Digitalis*), strophanthin (from *Strophanthus*).

6. **Saponins.** They are glycoside having steroids (*e.g.*, sapogenin) which form foam when their watery solution is shaken. Saponins are widely distributed in plants (*e.g.*, *Saponaria, Quillaja*). They can dissolve RBCs even in high dilution.

7. **Anabolic Steroids.** They are synthetic derivatives of testosterone which are used clinically in promoting growth and repair of body tissues in senile and debilitating illness.

Toad poisons and some carcinogenic hydrocarbons are also steroid in nature.

Cholesterol

Cholesterol ($C_{27}H_{45}OH$) is the common sterol found in many animals, human beings and

some plants (*e.g.*, Potato or *Solanum tuberosum*). It is manufactured inside the body in liver as well as got from outside. Cholesterol occurs in both free (Fig. 9.14) and combined form when it is esterified with a fatty acid. Cholesterol and its ester are insoluble in water. Therefore, when cholesterol level rises in blood, it tends to get deposited in the walls of arteries. This is known as atherosclerosis. It leads to high blood pressure and many heart diseases. Crystallisation inside gall bladder leads to formation of gall bladder stones.

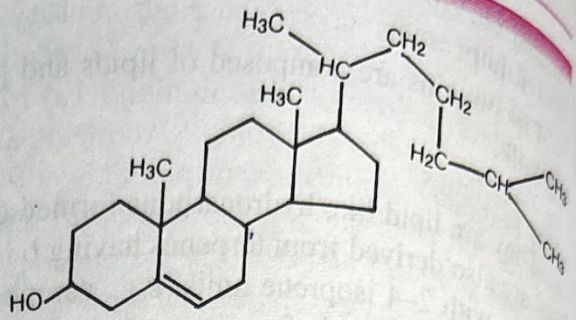


Fig. 9.14. Structure of Cholesterol.

Useful Role of Steroids

(i) Cholesterol is precursor of most steroid hormones like progesterone, estradiol (female sex hormones), testosterone (male sex hormone), aldosterone, cortisol (adrenal cortex hormones), ecdysone (moulting hormone). (ii) Steroid hormones control sex expression, sexual cycle, allergies, differentiation and body fluids. (iii) Cholesterol forms vitamin D on exposure to ultraviolet rays. (iv) Cholesterol is useful in absorption of fatty acids. (v) Steroid **diosgenin** obtained from *Dioscorea* (yam) has antifertility properties. (vi) Anabolic steroids are useful in correcting debilitating disorders. They are misused for body building by players. (vii) Sterols are known to be essential for plant growth, induction of flowering and expression of sex in some plants. (viii) Cholesterol is a constituent of animal cell membrane. (ix) Cholesterol helps in forming bile salts. (x) Cardiac glycosides tone up cardiac muscles and correct arrythmia. (xi) Birth control pills have steroid hormone progesterone with or without oestrogen.

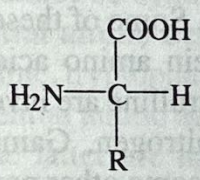
Functions of Lipids

(1) Fats serve as food reserve in both plants and animals. Hibernating animals store extra fat prior to onset of winter. Migratory birds also do so before migration. (2) They function as concentrated food because as compared to carbohydrates they yield more than twice as much energy per unit weight (9.3 kcal/gm : 4.5 kcal/gm). (3) Fats can be converted to carbohydrates. Therefore, fats stored in oil seeds (*e.g.*, Groundnut, Mustard, Castor, Sunflower, Cotton, Coconut) not only provide energy but also raw materials for growth of embryo. (4) In seeds and spores lipids help in thermal insulation, protection from ultraviolet radiations and loss of water. (5) Vitamin A, D, E and K are soluble in fats. The latter not only act as their carriers but also protect them from oxidation. (6) In animals fat occurs as droplets inside cells called **adipocytes**. Adipocytes of cold blooded or poikilothermic animals have higher amount of unsaturated fatty acids as compared to warm blooded or homoeothermic animals. Fatty or adipose tissue forms an insulating layer below the skin of animals for protection against low temperature. Whale has a very thick layer of subcutaneous fat called **blubber**. Animals of colder regions also have a thick fatty layer for insulation, *e.g.*, Polar Bear. (7) Subcutaneous fat rounds off the body contours of animals and human beings. In animals the fats produce a shock absorbing cushion around eye balls, gonads, kidneys and other vital organs. (8) Edible oils extracted from many seeds are used in cooking. Animal fats present in milk yield butter and ghee. (9) Plant oils are used as low cholesterol fat. They are also hydrogenated to form vegetable ghee. (10) Soap was previously manufactured from

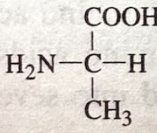
animal fat. Now-a-days plant fats are used for this purpose. (11) Drying oils having unsaturated fatty acids are used in paint industry. (12) Waxes form a protective layer over the animal fur. They protect the floating leaves of aquatic plants against wetting. In land plants they reduce the rate of transpiration. (13) Myelin sheath around nerve fibres takes part in insulation. (14) Phospholipids, glycolipids and sterols are components of cell membranes. (15) Fragrance of many plant products is due to fat-like substances called **terpenes**. (16) In birds, oil from preen gland is used to lubricate feathers and protect them from wetting. Hair are similarly lubricated in mammalian skin. It prevents their felting. The skin is also protected from drying up. (17) Desert animals employ fat as source of metabolic water, *e.g.*, Kangaroo Rat, Camel. Kangaroo or Desert Rat does not drink water. Camel uses fat stored in its hump for obtaining metabolic water during extreme desiccating conditions.

AMINO ACIDS

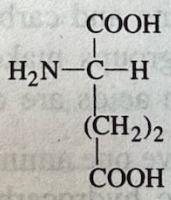
They are organic acids (with carboxylic group —COOH) having amino group (—NH_2) generally attached to α-carbon or carbon next to the carboxylic group. Carboxylic group provides an acidic property to the amino acid while amino group gives it a basic reaction. The α-carbon also bears a variable hydrocarbon or alkyl group R and hydrogen. Amino acids are, therefore, substituted methanes where the four substituent groups occupy the four valency positions.



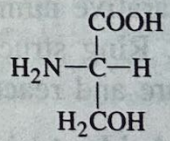
R is represented by H in glycine, methyl group in alanine, hydroxymethyl in serine. In others it may be straight or branched hydrocarbon chain or a cyclic group. The hydrocarbon may further be **polar** (*e.g.*, serine, glutamate or glutamic acid) or **nonpolar** (*e.g.*, alanine).



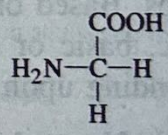
Alanine
(Nonpolar hydrocarbon)



Glutamate
(Polar hydrocarbon)

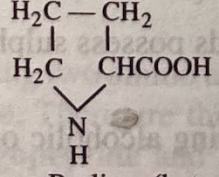


Serine
(polar hydrocarbon)

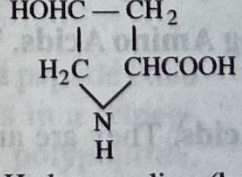


Glycine

In proline and hydroxyproline, amino group (—NH_2) is replaced by imino group (>NH) which also represents the tail of R-group. Proline and hydroxyproline are called heterocyclic amino acids.



Proline (heterocyclic)



Hydroxyporline (heterocyclic)

Except glycine, α–carbon is asymmetric and the amino acids are generally laevorotatory. Certain nonprotein amino acids are dextrorotatory.

Twenty types of amino acids and amides occur in proteins (Table 9.3). They are called protein amino acids. Incorporation of protein amino acids is controlled by triplet codes of

DNA/mRNA. A protein may also possess noncoded amino acids. The latter are called rare **amino acids**. Rare amino acids are derived from the coded ones through modifications, *e.g.*, hydroxyproline from proline, hydroxylysine from lysine (both in collagen). Amino acid names are abbreviated with the help of first three letters of the name. Changes are made for amino acid derivatives like Asn for Asparagine (an amide).

Table 9.3. Principal Protein Amino Acids

A. *Neutral*	1. Glycine (Gly)	D. *S-Containing*	12. *Cysteine* (Cys)
	2. Alanine (Ala)		13. Methionine (Met)
	3. Valine (Val)	E. *Alcoholic*	14. Serine (Ser)
	4. Leucine (Leu)		15. Threonine (Thr)
	5. Isoleucine (Ile)	F. *Aromatic*	16. Phenylalanine (Phe)
B. *Acidic*	6. Aspartic acid (Asp)		17. Tyrosine (Tyr)
	7. Asparagine (amide, Asn)		18. Tryptophan (Try)
	8. Glutamic acid (Glu)	G. *Heterocyclic*	19. Histidine (His)
	9. Glutamine (amide, Gln)		20. Proline (Pro)
C. *Basic*	10. Arginine (Arg)		
	11. Lysine (Lys)		

Non-protein amino acids are many in number. They occur both in the free state as well as in combined state, but not in proteins. Some of these are found in antibiotics and are called amino acid analogues. Other non-protein amino acids take part in important biosynthetic pathways. For example, ornithine and citrulline are involved in urea cycle. Homo-arginine and diaminobutyric acid are a source of nitrogen. Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter in brain and some other parts. Diaminopimelic acid is an intermediate of lysine synthesis.

All amino acids contain C, H, O and N. Some additionally contain sulphur. Physical and chemical properties of amino acids are mainly due to the amino, carboxyl and R functional groups. Based on comparative number of amino and carboxyl groups, amino acids can be acidic, basic or neutral. Ring structure in R-groups makes certain amino acids aromatic. Depending upon structure and reaction, amino acids are differentiated into seven types.

(a) **Neutral Amino Acids.** Amino acids have one amino group and one carboxylic group (mono-amino monocarboxylic) with noncyclic hydrocarbon chain, *e.g.*, glycine, alanine, valine.

(b) **Acidic Amino Acids**. The amino acids have an extra carboxylic group (mono-amino dicarboxylic), *e.g.*, glutamate (glutamic acid), asparate (aspartic acid).

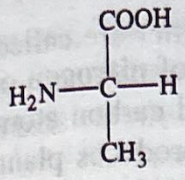
(c) **Basic Amino Acids**. They have an additional amino group without forming amides (diamino monocarboxylic), *e.g.*, arginine, lysine.

(d) **Sulphur Containing Amino Acids**. The amino acids possess sulphur, *e.g.*, cysteine, methionine.

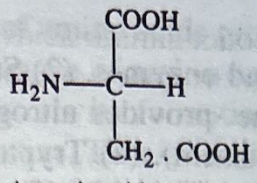
(e) **Alcoholic Amino Acids**. They are amino acids having alcoholic or hydroxyl group, *e.g.*, serine, threonine.

(f) **Aromatic Amino Acids**. They possess cyclic structure with a straight side chain bearing carboxylic and amino group, *e.g.*, phenylalanine, tryptophan (actually heterocyclic) or tyrosine (having OH group).

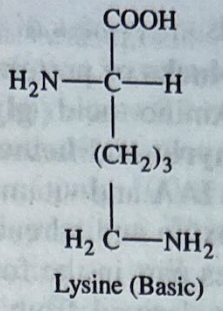
(g) **Heterocyclic Amino Acids**. They have nitrogen in the ring structure, *e.g.*, histidine, proline.



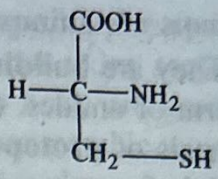
Alanine (neutral)



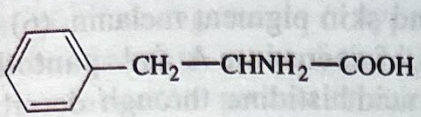
Aspartic acid (acidic)



Lysine (Basic)

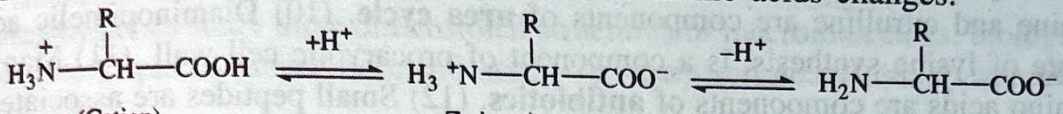


Cysteine (Sulphur containing)



Phenylalanine (Aromatic)

A particular property of amino acids is the ionizable nature of NH_2 and COOH groups. Hence in solutions of different *p*H, the structure of amino acids changes.



(Cation) Zwitter ion (anion)

Plants can synthesise all the protein amino acids required by them. Animals cannot manufacture their own amino acids from raw materials. For this they are dependent upon the plants directly or indirectly. Animals, however, have the ability to form several amino acids through transformation or transamination. They cannot synthesise seven of the protein amino acids. The amino acids which cannot be synthesised by animals through transformation or transamination are called **essential amino acids**. These amino acids must be present in their diet, *viz*., leucine, isoleucine, valine, tryptophan, phenylalanine, lysine and methionine. Human adults require an additional essential amino acid named threonine while children need two more (arginine and histidine). The latter are named as **semi-essential amino acids**.

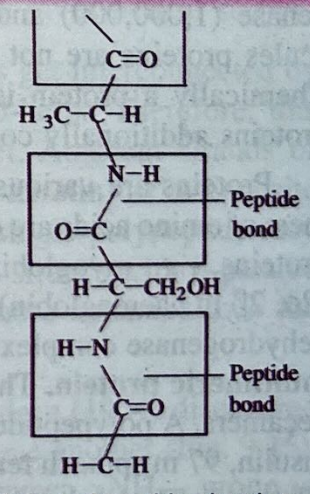
Differences between Essential and Nonessential Amino Acids

Essential Amino Acids	*Nonessential Amino Acids*
1. They are those amino acids which the human body cannot synthesise from raw materials.	1. They are amino acids which can be synthesized by human body from raw materials.
2. They are seven in number.	2. They are 13 in number but one in adults and two in children are slow to be formed.
3. Essential amino acids are obtained from dietary proteins.	3. They need not be present in the diet.

Peptide Formation

Amino acids condense to produce peptides. Generally condensation reaction occurs between a primer amino acid or peptide and another amino acid. Molecule of water is eliminated. The bond thus formed —NHCO— is actually **amide bond** which is popularly known as **peptide bond** or linkage.

Chain containing two amino acids linked by a peptide bond is called **dipeptide**. There are three amino acids in a **tripeptide**, a few in **oligopeptide** and numerous in a **polypeptide**. The term peptide is more commonly used instead of oligopeptide. Like an amino acid a peptide has carboxylic group at one end and amino group at the other end. Occasionally the peptide is acidic or basic due to the presence of specific amino acids.



A part of peptide showing peptide bonds linking amino acids.

Functions of Amino Acids

(1) They are **building blocks** of proteins and enzymes. (2) **Storage** of **nitrogen** occurs in the form of amides. (3) Amino acid **glycine** provides nitrogen and carbon atoms for the synthesis of **protoporphyrin** and **heme** (= haem). (4) Tryptophan produces plant hormone indole 3-acetic acid or **IAA** and vitamin **nicotinamide**. (5) Amino acid **tyrosine** gives rise to two **hormones** (thyroxine and adrenaline) and skin pigment melanin. (6) **β-alanine**, a derivative of asparate, takes part in the formation of **coenzyme A** and **pantothenic acid** (a vitamin). (7) **Histamine** is derived from amino acid histidine through decarboxylation. Histamine is required for optimum functioning of muscles, blood capillaries and gastric secretion but in excess it causes anaphylaxis. (8) Some amino acids act as **donors of methyl group** in the synthesis of various organic compounds including alkaloids, *e.g.*, methionine. (9) Ornithine and citrulline are components of **urea cycle**. (10) Diaminopimelic acid, an intermediate of lysine synthesis, is a component of procaryotic cell wall. (11) Many non-protein amino acids are components of **antibiotics**. (12) Small peptides are associated with structural units of procaryotic wall called **peptidoglycans** (= mucopeptides). (13) **Enkephalins** are pentapeptides produced by nerve cells. They function as body opiates influencing perception of pleasure and pain. (14) Excess amino acids are deaminated in liver. The organic acids are changed to glucose (**gluconeogenesis**) or used directly in metabolism.

PROTEINS

Proteins (Gk. *proteios*– of the first rank, Berzelius, 1838) are large-sized **heteropolymeric** macromolecules having one or more polypeptides (chains of amino acids). The term polypeptide is often used interchangeably with protein. However, a single polypeptide must be at least 50 amino acid long in order to qualify for the term. Proteins are body builders of organisms. They are the most abundant and most varied of the macromolecules of the cells which constitute about 50% of their dry weight. A bacterial cell has 1000–2000 types of proteins. Each type of cell has some unique proteins. Closely related species have many similar proteins while unrelated species have fewer common proteins. Thus protein proximity shows evolutionary relationship.

The minimum molecular weight of a protein is that of adrenocorticotropin hormone (4500)*, insulin (bovine insulin – 5733) and bacterial ferredoxin (about 6000). Other common proteins are human haemoglobin (66500), enzyme urease (483000), iso-citrate dehydrogenase (1,000,000) and pyruvate dehydrogenase complex (4,600,000). Being macromolecules proteins are not freely soluble in water but form colloidal complex with the same. Chemically a protein is made of carbon, hydrogen, nitrogen, oxygen and sulphur. Some proteins additionally contain phosphorus, iron and other elements.

Proteins are variously folded linear **heteropolymers of amino acids**. The linear polymers of amino acids are called polypeptides. A protein may have one polypeptide (monomeric proteins, *e.g.*, myoglobin, ribonuclease), two polypeptides (*e.g.*, insulin), four polypeptides (2α, 2β in haemoglobin) or more (24 in ribulose biphosphate carboxylase and 72 in pyruvate dehydrogenase complex). A protein having two or more polypeptides is called **multimer** or **multimeric protein**. The common multimers are dimers, trimers, tetramers, pentamers and decamers. A polypeptide contains from a few (21 and 30 in the two polypeptides of human insulin, 97 in spinach ferredoxin) to a few hundred amino acid residues (582 in human serum albumin). The amino acids are linked serially by **peptide bonds** (—CONH—) formed between amino group ($—NH_2$) of one amino acid and carboxylic group (—COOH) of the

*4600 according to one calculation.

adjacent one. The sequence of amino acids present in a polypeptide is specific for a particular protein. The distinctive sequence of amino acid units is governed by the codon sequence of the gene or cistron that controls its formation. Only some 20 amino acids are used in the synthesis of all types of proteins. This is similar to the formation of innumerable words from a limited number of alphabets. In a polypeptide of only 100 amino acid residues, there is possibility of 20^{100} arrangements or types of polypeptides. This accounts for thousands of specific proteins found in each of the living species as well as those of the past species. **Collagen** is most abundant protein of animal world. **Rubisco** (ribulose biphosphate carboxylase– oxygenase) is not only the most abundant protein in plants but also the whole biosphere.

Organisation (Levels of Structure)

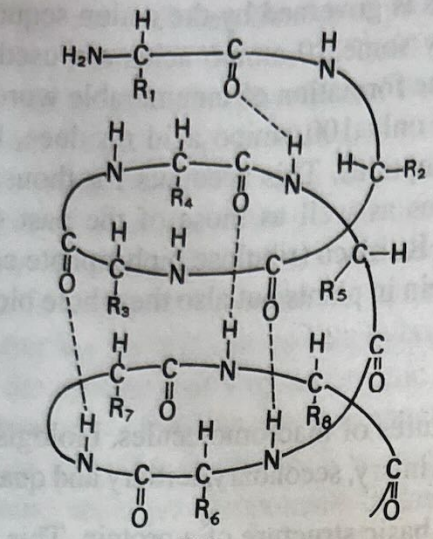
While physicists study three dimensional structures of macromolecules, biologists study protein structure upto four levels of organisation— primary, secondary, tertiary and quarternary.

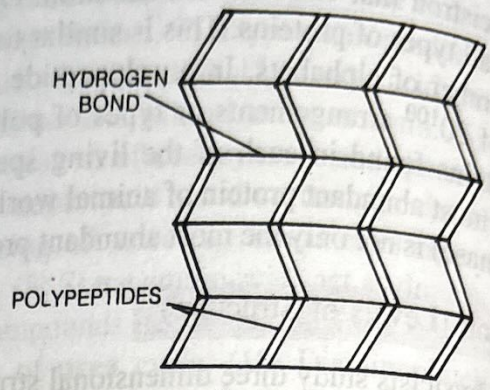
1. **Primary Structure**. It is the description of basic structure of a protein. This includes number of polypeptides, number and sequence of amino acids in each polypeptide. The latter is determined genetically (by DNA) through transcription and translation. Specific amino acids determine the places where polypeptides are to bend or fold and where the different lengths will be attracted to each other. The distance between two adjacent peptide bonds is about 0.35 nm. Conventionally, the left end of the protein primary structure is represented by the first amino acid while the right end is represented by last amino acid.

2. **Secondary Structure** (Fig. 9.15). It is the development of new stearic relationships of amino acids present in the linear sequence inside the polypeptides. Some of the new relationships are of regular nature and give periodicity to the structure. There are three types of secondary structures— α-helix, β-pleated and collagen helix. The α and β terms simply designate the first and the second type of secondary structures discovered in proteins. In α-helix the polypeptide chain is coiled spirally, generally in right handed manner. At places the helix is less regular, forming random coils. The helix is stabilized by hydrogen bonds between oxygen of carboxylic group (— CO group) of one amino acid residue and >NH group of next fourth amino acid residue. Actually all the main chain —CO and >NH groups are hydrogen bonded. α-helical coiled secondary structure is found in several proteins, *e.g.*, keratin (hair), myosin, tropomyosin (both muscles), epidermin (skin), fibrin (blood clot). Two or more polypeptides can further coil around each other to form cables. This gives helical strand.

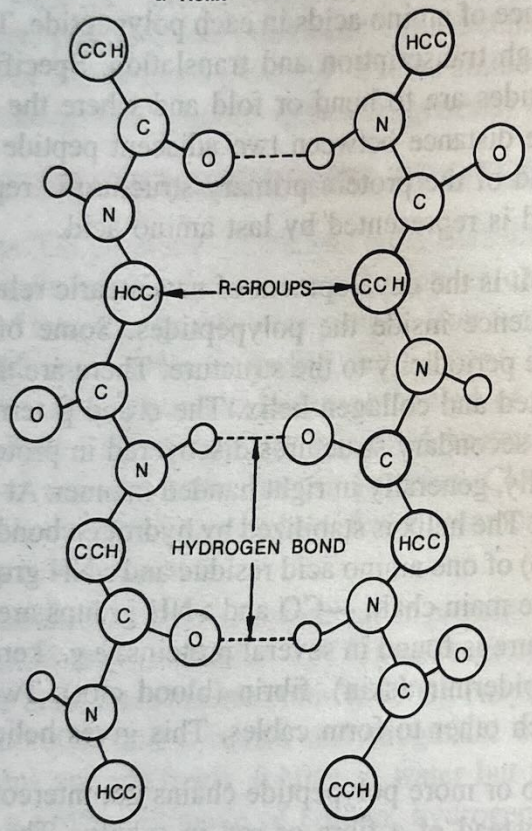
In **β-pleated** secondary structure two or more polypeptide chains get interconnected by hydrogen bonds. A sheet is produced instead of a fibre or rod in α-helix. Therefore, this secondary structure is often called pleated sheet or β-pleated sheet. Adjacent strands of polypeptides may run in the same direction (parallel β-sheet, *e.g.*, β-keratin) or in opposite directions (antiparallel β-sheet, *e.g.*, fibroin of silk). In some cases single polypeptide may show α-helix in some portion and bent to form two or more parallel strands with β-pleated structure in other parts, *e.g.*, ribonuclease. β-pleated proteins are more extended than the ones having α-helix.

In collagen (the most abundant protein in our body), Ramachandran (1954) discovered that there are generally three strands or polypeptides coiled around one another (Fig. 9.15). The coil is strengthened by the establishment of hydrogen bond between >NH— group of glycine residue of each strand with —CO group of the other two strands. There is also a locking effect with the help of proline and hydroxyproline residues.

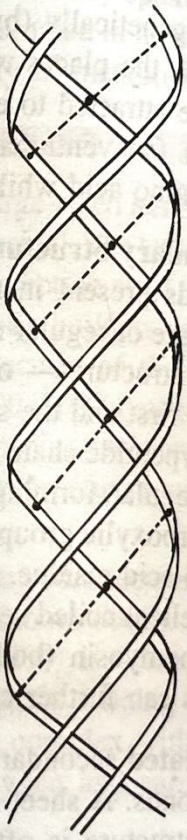




β -Pleated (DIAGRAMMATIC)



Extended β -pleated (ANTIPARALLEL) POLYPEPTIDE STRANDS



Collagen Helix

Fig. 9.15. Secondary structure of proteins.

3. **Tertiary Structure**. There is bending and folding of various types to form spheres, rods or fibres. It further brings new stearic relationships of amino acids specially those which are far apart in the linear sequence. The active sites (*e.g.*, polar side chains) of the protein are often brought towards the surface. Certain other side chains (*e.g.*, hydrophobic) are brought to the interior of the protein. Tertiary structure is stabilized by several types of bonds— hydrogen bonds, ionic bonds, van der Waal's interactions, covalent bonds, hydrophobic bonds (Fig. 9.16). Tertiary structure gives the protein a three dimensional conformation (Fig. 9.17).

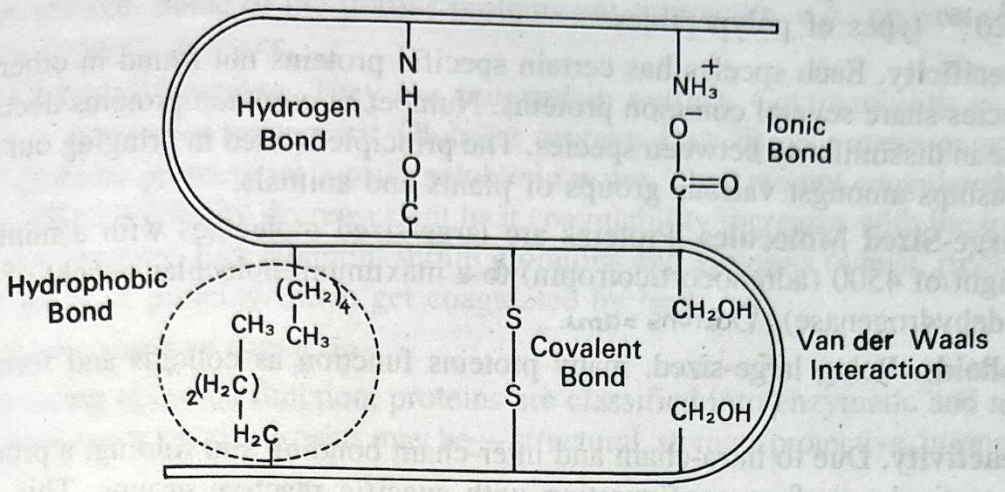


Fig. 9.16. Various types of bonds or interactions found during the coiling of polypeptide.

In protein structure, **covalent bonds** are the strongest. They are of two types, peptide bonds and —S—S— (disulphide) bonds. **Ionic bonds** or electrostatic bonds occur due to attractive force between oppositely charged ionised groups *e.g.*, $—NH_3^+$ and $—COO^-$. **Hydrogen bonds** develop due to sharing of H^+ or proton by two electronegative atoms. **van der Waals interactions** develop with charge fluctuations between two closely placed groups (*e.g.*, $—CH_2OH$ and $—CH_2OH$). **Hydrophobic bond** is formed between two nonpolar groups. It helps in excluding water in that area and increasing compaction.

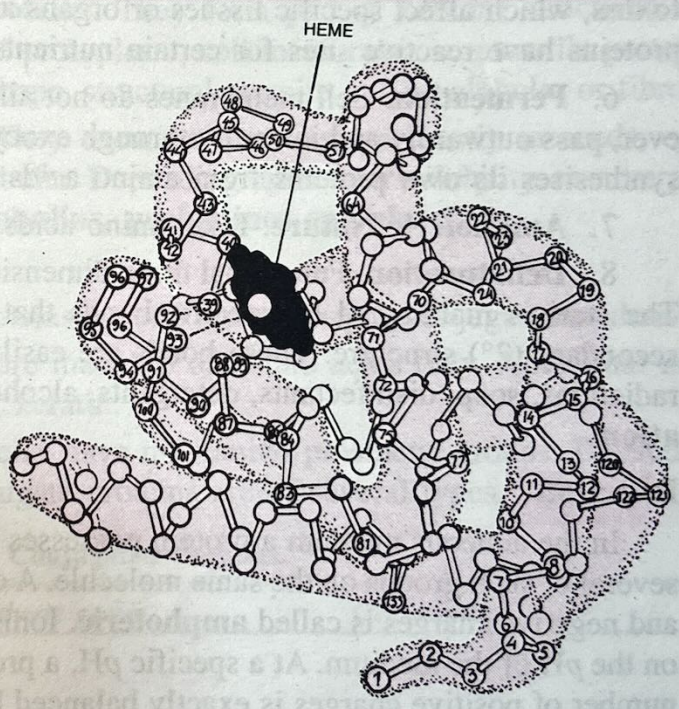


Fig. 9.17. Tertiary structure of protein myoglobin.

The bonds required to form tertiary structure can be easily broken by high energy radiations, high temperature, drastic changes in *p*H and salts of heavy metals. This process of degrading the tertiary structure is known as **denaturation**. Proteins can also be precipitated or coagulated by several chemicals and low temperature. In some cases removal of denaturing agent causes re-establishment of the bonds required for maintenance of tertiary structure. The phenomenon is called **renaturation.**

4. **Quarternary Structure**. This is found only in multimeric proteins. Each polypeptide develops its own tertiary structure and functions as subunit of the protein. The different subunit chains fit or pack together to give the conformation, *e.g.*, haemoglobin (four polypeptides, 2α and 2β).

Properties

1. **Variety**. There are thousands of proteins present in each organism. This large variety

is due to specific arrangement of amino acids in their chains. For example, 100 amino acids can form 20^{100} types of polypeptides.

2. **Specificity**. Each species has certain specific proteins not found in others. Closely related species share several common proteins. Number of common proteins decreases with the increase in dissimilarity between species. The principle is used in bringing out evolutionary relationships amongst various groups of plants and animals.

3. **Large-Sized Molecules**. Proteins are large-sized molecules with a minimum molecular weight of 4500 (adrenocorticotropin) to a maximum molecular weight of 4,600,000 (pyruvate dehydrogenase). Daltons = amu

4. **Colloids**. Being large-sized, many proteins function as colloids and form colloidal solution.

5. **Reactivity**. Due to intra-chain and inter-chain bonding and folding, a protein comes to have a particular surface configuration with specific reactive groups. This specificity provides functional diversity to proteins for performing different cellular activities. **Enzymes** are specific due to their proteinaceous nature. **Antibodies** are complex glycoproteins which attach to particular pathogens and their toxins for their immobilisation. Certain bacterial **toxins**, which affect specific tissues or organs are also proteins. Similarly membrane based proteins have reactive sites for certain nutrients.

6. **Permeation**. Cell membranes do not allow permeation to proteins. They can, however, pass outwardly and inwardly through exocytosis and endocytosis. Normally, every cell synthesises its own proteins from amino acids.

7. **Amphoteric Nature**. Like amino acids, proteins are amphoteric.

8. **Denaturation**. Functional three-dimensional form of a protein is called **native state**. The state is maintained by specific bonds that form its quaternary (4°), tertiary (3°) and secondary (2°) structure. These bonds are easily broken by high temperature, high energy radiations, soap, disinfectants, detergents, alcohol, etc. The phenomenon is called **denaturation**.

Reaction

In the aqueous medium a protein possesses both cationic and anionic groups. There are several of such groups on the same molecule. A chemical, like protein, carrying both positive and negative charges is called **amphoteric**. Ionisation of cationic or anionic groups depends on the *p*H of the medium. At a specific *p*H, a protein may be electrically neutral because the number of positive charges is exactly balanced by the number of negative charges. This *p*H is known as **isoelectric point**. At physiological *p*H of 7.4, a protein may have more of negative charges. Such proteins are called **basic proteins**. They are rich in basic amino acids like lysine and arginine. Histones associated with DNA are basic proteins. **Acidic proteins** have more of positive charges. They are rich in acidic amino acids like aspartic acid and glutamic acid. Most of the blood proteins are acidic in nature. There is a third category of **neutral proteins** which have their isoelectric point at 7.4 *p*H.

Types

Proteins are classified on the basis of their shape, function and constitution :

(a) *On the Basis of Shape*

Depending upon their shape, proteins are divided into two categories, fibrous and globular.

1. **Fibrous Proteins**. They are thread like proteins which may occur singly or in groups. They are tough, non-enzymatic and structural proteins. Fibrous proteins generally

possess secondary structure. They are insoluble in water. Keratin of skin and hair is such a fibrous protein. Some of the fibrous proteins are contractile, *e.g.*, myosin of muscles and elastin of connective tissue.

2. **Globular Proteins**. They are rounded in outline. Contractibility is absent. Final structure is tertiary or quaternary. Globular proteins may be enzymatic or non-enzymatic. Smaller globular proteins are mostly soluble in water. They are not coagulated by heat, *e.g.*, histones. Water solubility decreases but heat coagulability increases with the increase in size of globular proteins. Egg albumin, serum globulins and glutelins (wheat, rice) are examples of large globular proteins which get coagulated by heat.

(b) *On the Basis of Function*

Depending upon the function, proteins are classified into enzymatic and non-enzymatic types. The non-enzymatic proteins may be— structural, storage, protective, hormonal, transport, types.

1. **Enzymatic Proteins**. They are proteins which function as enzymes, either directly (*e.g.*, amylase, pepsin) or in conjunction with a non-protein part called cofactor (*e.g.*, dehydrogenases). Enzymatic proteins are usually globular in shape.

2. **Structural or Protoplasmic Proteins**. They form part of cellular structures and their products, *e.g.*, colloidal complex of protoplasm, cell membranes, contractile proteins, structural proteins of hair and nails. In shape, structural proteins can be globular or fibrous.

3. **Reserve or Storage Proteins**. They occur as food reserve mostly in seeds, eggs or milk. Storage proteins are usually globular. Depending upon their solubility, reserve proteins are of four types— **albumins, globulins, prolamines** and **glutelins**.

(c) *On the Basis of Constitution*

On the basis of their constitution, proteins are of 3 types– simple, conjugate and derived.

1. **Simple Proteins**. The proteins are made up of amino acids only. Additional non-amino groups are absent, *e.g.*, histones, keratin.

2. **Conjugated Proteins**. The proteins have non-amino prosthetic groups. Depending upon the type of prosthetic group, conjugate proteins are of several types.

Table 9.4. Conjugated Proteins.

Conjugated Proteins	*Prosthetic group*	*Occurrence*
(i) **Nucleoproteins**		
(a) Deoxyribonucleoproteins	DNA	Chromosomes
(b) Ribonucleoproteins	RNA	Ribosomes
(ii) **Chromoproteins**		
(a) Flavoproteins	FMN, FAD	Enzyme
(b) Haemoglobin	Fe- Haem	Erythrocytes
(c) Cytochromes	Fe- Heam	Enzymes, Electron carriers
(d) Rhodopsin	Vitamin A	Retina
(e) Haemocyanin	Cu- Haem	Blood of some animals
(f) Chloroplastins	Chlorophylls	Thylakoids
(g) Biliproteins	Phycobilins	Phycobilisomes
(iii) **Metalloproteins**	Zn, Mn, Cu, Fe	Iron in ferritin
(iv) **Glycoproteins**	Carbohydrates	Plasma membrane, Cell wall
(v) **Mucoproteins**	Mucoid carbohydrates	Mucin (saliva)

(vi) **Lipoproteins**	Lipids	Cell membranes
(vii) **Phosphoproteins**	Phosphate	Casein (milk), Vitellin (egg)

3. **Derived Proteins**. They are got from proteins through denaturation, coagulation and breakdown, *e.g.*, metaprotein, proteoses, fibrin. Precursor of fibrin is an elongated but asymmetric soluble plasma protein called fibrinogen.

(d) *On the Basis of Quality*

1. **Complete Proteins.** They are proteins which contain all the essential amino acids required by humans, *e.g.*, milk, egg, meat, fish. Soya also possesses a nearly complete protein. Complete proteins are also called **first class proteins**.

2. **Incomplete Proteins.** They are proteins which lack one or more essential amino acids. Most of the plant proteins are incomplete proteins. Plant proteins, being incomplete, are also called **second class proteins.**

Functions

1. **Protective Structures**. Fibrous protein **keratin** forms external protective structures of animals like nails, hoofs, scales, hair, feathers, horny layer of skin, etc. Silkworms protect themselves in the cocoon stage by silk fibres. The latter are built up of protein **fibroin**. Spider webs are also built of this protein.

Table 9.5. Some Proteins and their functions.

Protein	*Functions*
Collagen	Intercellular ground substance
Trypsin	Enzyme
Insulin	Hormone
Antibody	Fights infectious agents
Receptor	Sensory reception (smell, taste, hormone, etc.)
GLUT-4	Enables glucose transport into cells

2. **Defence Proteins**. Immunoglobulins or antibodies are proteins produced by lymphocytes. They are meant for recognising and neutralizing foreign proteins, toxins, viruses and other pathogens.

3. **Toxins**. They are both defensive and offensive proteins produced by certain animals, bacteria and some plants, *e.g.*, snake venom, ricin of castor, bacterial toxins, etc.

4. **Structural Proteins**. Proteins constitute more than 50% of the dry weight of protoplast. They take part in formation of colloidal complex of protoplast, cell organelles, cell membranes and cell products. Many proteins form supporting structures, *e.g.*, elastin of ligaments, collagen of tendons, cartilage, bone and connective tissue. Along with proteoglycans*, the two above mentioned fibrous proteins form a major component of connective tissue.

5. **Compatability Proteins**. Pollen grains possess specific proteins in their walls for compatibility-incompatibility reaction with the stigma during pollination.

6. **Contractile Proteins**. Actin and myosin are fibrous proteins which form the contractile system of muscles. Contractile system is basically made up of protein actin but association with myosin is essential for contraction.

7. **Microtubules**. They are unbranched, hollow, submicroscopic tubules which form the structural material of cilia, flagella, basal bodies, centrioles and spindle apparatus. Microtubules are built up of protofilaments formed of two related proteins, α and β-tubulins.

8. **P-Protein**. It is a special protein present in sieve tube elements. The protein is vibratile and is believed to actively participate in the transport of nutrients.

9. **Enzymes**. There are over 2000 enzymes. Except a few, all others are built up of proteins alone or in conjugation with some non-protein materials called cofactors. Enzymes catalyse all the chemical reactions that occur in the living world, *e.g.*, pepsin, trypsin, ribonuclease, flavoprotein, etc.

10. **Transport Proteins (Carrier Proteins).** Haemoglobin of RBCs transports oxygen from lungs to different parts of the body. Myoglobin of muscles stores oxygen. α-globulin of

*Proteoglycans are mucopolysaccharides hybridised with small quantity of protein (about 5%) *e.g.*, chondroitin sulphate, keratan sulphate, hyaluronate.

blood carries thyroxine and bilirubin. β-globulin transports vitamins A, D and K, cholesterol and ions. Albumin carries calcium and fatty acids. A number of carrier proteins occur in the cell membranes for transporting specific materials to the inside, *e.g.*, glucose, amino acids.

11. **Storage Proteins**. They occur in milk, eggs and seeds to nourish the young ones. Important protein of milk is **casein**. Major protein of egg white is **ovalbumin** while it is **glutelin** in cereals. Iron storing protein commonly found in animal tissue is **ferritin**. Proteins having all the essential amino acids are called **first class proteins**.

12. **Receptor Proteins**. They occur on the external surface of cell membranes. The proteins bind to specific information molecules like hormones and mediate in the cellular effects, generally through the formation of either cAMP or specific activators. Receptors are specific for different hormones, *e.g.*, adrenaline receptor, β-adrenaline receptor (epinephrine receptor).

13. **Protein Buffers**. The polar side chains of the proteins can combine with excess acid and base. This helps in keeping the *p*H constant.

14. **Hormones**. Some hormones are proteinaceous, *e.g.*, insulin (regulates sugar metabolism), growth hormone of pituitary, parathyroid hormone parathormone (regulates Ca and phosphate transport).

15. **Repressors**. Most of the repressors of the genes are proteinaceous in nature. They regulate gene action.

16. **Sweet Proteins.** Monellin (from *Dioscoreothyllum cumminsii*), thaumitin (Katempe fruit *Thaumatococcus daniellii*) and brazzein (from *Pentadiplandra brazzeana*) are sweetest chemicals which are natural proteins. They are some 2000 times as sweet as sugar. Their use can be boon to diabetic patients since they are nonfattening, noncaloric and nontoxic sweeteners.

17. **Blood Clotting Proteins**. Fibrinogen and thrombin prevent blood loss from injured vessels by causing clotting of blood.

18. **Antibodies**. They are formed of proteins.

19. **Visual Pigments**. Rhodopsin and iodopsin are protein pigments respectively present in rods and cones of retina. They take part in perception of image.

20. **Mucoproteins**. They are produced by salivary glands and mucous glands of alimentary canal. Mucoproteins form mucus. Mucus protects the lining layer of alimentary canal from friction and digestive juices, *e.g,* gastric mucoprotein is resistant to pepsin digestion.

21. **Other Proteins. Nucleoproteins** are proteins associated with nucleic acid. Human memory is believed to be stored in specific proteins called **memory proteins**. Proteins being multivalent macromolecules can carry on a number of chemical reactions.

NUCLEOTIDES

Nucleotides are basic units of nucleic acids. They also form energy carriers. Certain nucleotides act as coenzymes. Some nucleotides function as chemical messengers.

A nucleotide is a condensation product of three chemicals— a pentose sugar, phosphoric acid and a nitrogen base. Two types of pentose sugars occur in nucleotides— ribose ($C_5H_{10}O_5$) and deoxyribose ($C_5H_{10}O_4$). Both of them occur in furanose state or pentagon ring with one oxygen and four carbon atoms. The fifth carbon having —CH_2OH complement lies outside the ring. Carbon atom 2 contains two hydrogen atoms in deoxyribose. It is hence called 2′ deoxyribose. Ribose differs from deoxyribose in having a hydroxyl (—OH) group instead of a second hydrogen at carbon 2 (Fig. 9.4). Deoxyribose occurs only in those nucleotides which make up the hereditary material DNA. Ribose is found in nucleotides forming RNA and a number of other compounds like AMP, ATP, NAD, NADP, FAD, CoA, etc.

Nitrogen bases are **heterocyclic compounds**. They are of two types, **substituted, purines** and **substituted pyrimidines**. Pyrimidines are 6-membered rings while purines are 9-membered double rings. A pyrimidine ring has nitrogen at 1 and 3 positions. A purine has

imidazole ring joined to pyrimidine ring at 4 and 5 positions. It has nitrogens at 1, 3, 7 and 9 positions. There are two types of purines— **adenine** (A) and **guanine** (G). Pyrimidine bases are of three types— **cystosine** (C), **thymine** (T) and **uracil** (U).

Differences between Purine and Pyrimidine

Purine	*Pyrimidine*
1. Purines are larger-sized nitrogen containing biomolecules.	1. Pyrimidines are smaller-sized nitrogen containing biomolecules.
2. A purine is nine-membered.	2. A pyrimidine is 6-membered.
3. It is a double ring.	3. It is a single ring.
4. A purine contains four nitrogen atoms at 1, 3, 7 and 9 positions.	4. A pyrimidine has nitrogen atoms at two places, 1 and 3 positions.
5. Purine bases are of two types, adenine (A) and guanine (G).	5. Pyrimidine bases are of three types— cytosine (C), thymine (T) and uracil (U).

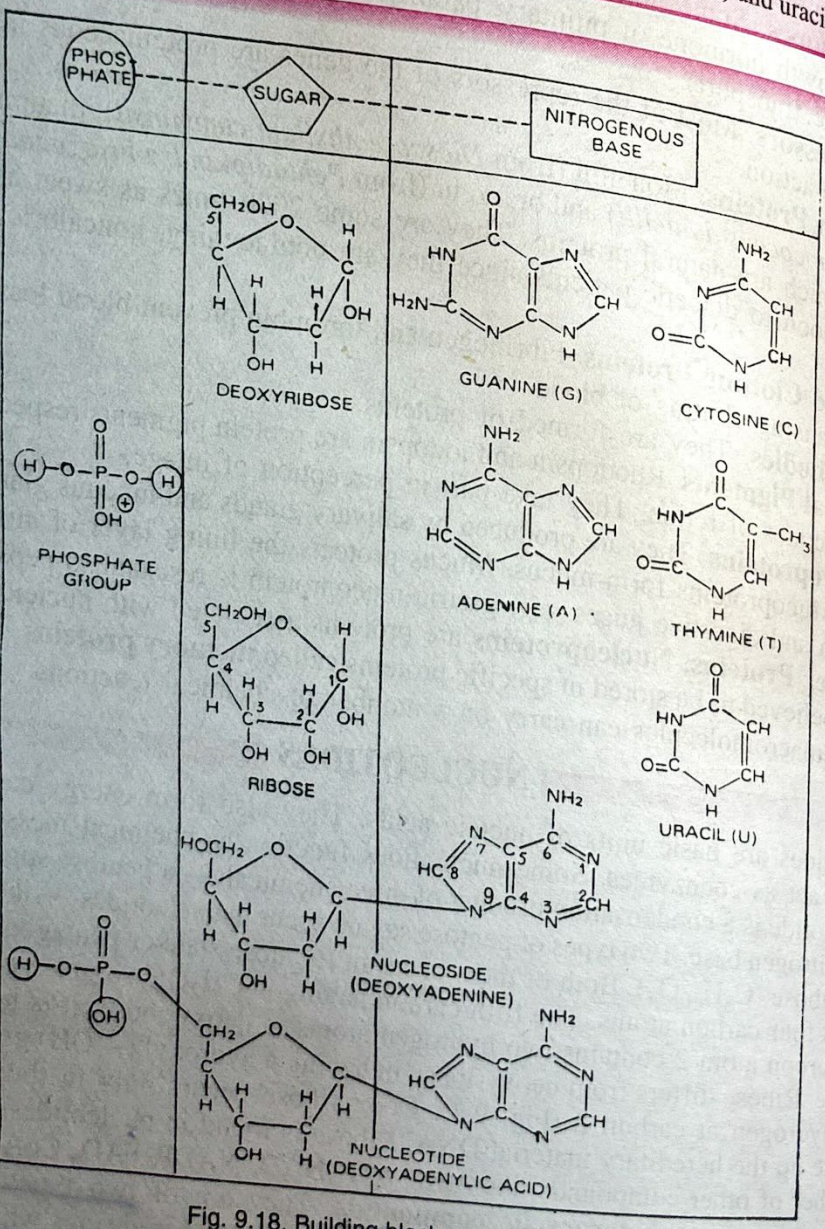


Fig. 9.18. Building blocks of nucleic acids.

A combination of a nitrogen base with a pentose sugar is known as **nucleoside**. The nitrogen base combines with the sugar molecule at its carbon atom 1′ in a glycosidic bond (C–N–C) by one of its nitrogen atoms (usually 1 in pyrimidines and 9 in purines). Depending upon the type of pentose sugar, nucleosides are differentiated into **ribonucleosides** and **deoxyribonucleosides**. Uracil produces nucleoside with only ribose sugars. Thymine similarly forms nucleoside with only deoxyribose sugar. Other nitrogen bases produce nucleosides with both ribose and deoxyribose sugars. The different nucleosides are **adenosine** (adenine +ribose), **deoxyadenosine** (adenine + deoxyribose), **guanosine** (guanine + ribose), **deoxyguanosine** (guanine + deoxyribose), **uridine** (uracil + ribose), **deoxythymidine** (thymine + deoxyribose), **cytidine** (cytosine + ribose), **deoxycytidine** (cytosine + deoxyribose).

Nucleotides are phosphoric acid esters of nucleosides. Phosphate combines with sugar molecule at its 5′ carbon atom. However, phosphate linked to 3′ carbon atom and rarely 2′ carbon atom is also known. The number of phosphoric or phosphate residues may be upto three in free occurring nucleotides. On the basis of pentose sugar present, nucleotides are distinguished into **ribonucleotides** (= ribotides) and **deoxyribonucleotides** (= deoxyribotides). Being phosphorylated form of nucleosides, they are also called nucleoside phosphates. The various nucleotides are adenylic acid (adenosine monophosphate or AMP), deoxyadenylic acid (dAMP), guanylic acid (guanosine monophosphate or GMP), deoxyguanylic acid (dGMP), uridylic acid (uridine monophosphate or UMP), deoxythymidylic acid (deoxythymidine monophosphate or dTMP), cytidylic acid (cytidine monophosphate or CMP) and deoxycytidylic acid (dCMP).

Differences between Nucleoside and Nucleotide

Nucleoside	*Nucleotide*
1. Nucleoside is a compound formed by the union of a nitrogen base with a pentose sugar.	1. Nucleotide is a compound formed by the union of a nitrogen base, a pentose sugar and phosphate.
2. It is a component of nucleotide.	2. Nucleotide is formed through phosphorylation of nucleoside.
3. It is slightly basic in nature.	3. A nucleotide is acidic in nature.

Higher Nucleotides

Nucleotides having more than one phosphate group are called **higher nucleotides**. Higher nucleotides occur in the free state. The second and third phosphates of higher nucleotides are attached against forces of repulsion between similarly charged phosphate radicals. Hence, the bonds attaching second and third phosphates are **high energy bonds**, much higher than common covalent bonds. A number of higher nucleotides are known— GDP, GTP (guanosine triphosphate), CDP, CTP (cytidine triphosphate), UDP, UTP (uridine triphosphate), ADP (adenos-

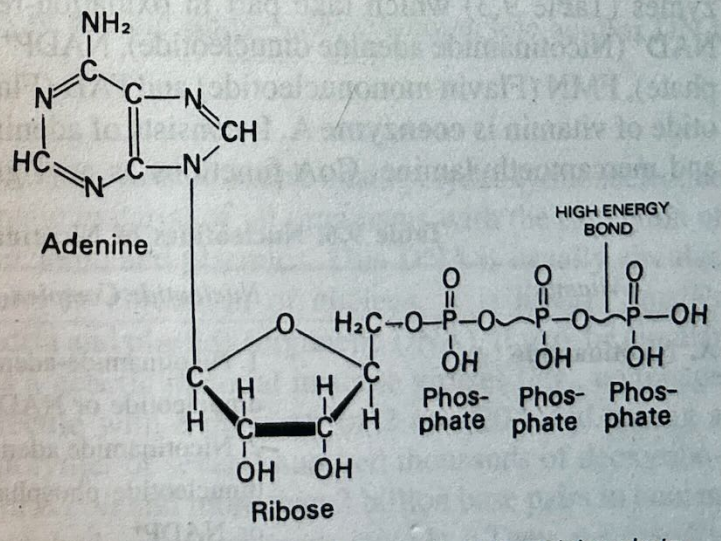


Fig. 9.19. Structure of ATP or adenosine triphosphate.

ine diphosphate) and ATP (adenosine triphosphate). Out of these ADP and ATP are well known. Due to the presence of high energy bonds, higher nucleotides function as **energy carriers**.

ATP. It is adenosine triphosphate. Adenosine triphosphate is formed of an adenine (a purine), a ribose (pentose sugar) and a row of three phosphate radicals attached to ribose. The complex formed by adenine and ribose is called **adenosine**. ATP or adenosine triphosphate (Fig. 9.19). was discovered by Karl Lohmann in 1929 from muscles. Its functioning was found out by Fritz Lipmann (1941). Lipmann is also called **father of ATP cycle**. The second and third phosphates of ATP are attached by **high energy bonds** (~P~P; Lipmann, 1941). The bonds have **high transfer potential** because they are held against great electrostatic repulsion.

ADP + Pi + Energy $\rightleftharpoons$ ATP reaction can occur in a cyclic manner of build up and breakdown reactions. It is an instant store house as well as source of energy. ATP is found in all living cells. It is also mobile within the cell. Therefore, ATP can pick up energy where it is being liberated and supply the same where it is being consumed. ATP is called **universal energy carrier** as well as **energy currency** of the cell. ATP usually obtains energy during respiration. It provides energy to cellular constituents for overcoming entropy and carrying out various activities like membrane transport, nerve conduction, muscle contraction, biosynthesis, microtubule synthesis, cytoplasmic streaming, cell movement, cell division, osmosis, heat, etc.

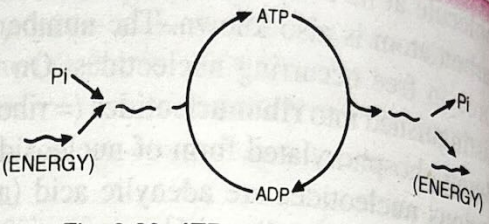


Fig. 9.20. ATP as energy currency (Energy is represented as a curved arrow).

The last phosphate bond is easily built up and broken for this purpose (Fig. 9.20). The enzyme is ATP-ase. Breakage of this bond releases 8.9 Kcal (8900 calories) per mole (older estimate 7.3 kcal/mole). Similar amount of energy is required for its building. The second energy rich bond has an energy equivalent of 6.5 Kcal per mole.

Nucleotides of Vitamins (Coenzymes)

Nicotinamide and flavin parts of riboflavin, two B-complex vitamins, function as nitrogen bases. Singly or in combination with other nucleotides, they produce important coenzymes (Table 9.3) which take part in oxidation-reduction reactions. These coenzymes are NAD^+ (Nicotinamide adenine dinucleotide), $NADP^+$ (Nicotinamide adenine dinucleotide phosphate), FMN (Flavin mononucleotide) and FAD (Flavin adenine dinucleotide). Another nucleotide of vitamin is **coenzyme** A. It consists of adenine, ribose, 3 phosphates, pantothenic acid and mercaptoethylamine. CoA functions as acyl group carrier, *e.g.*, Acetyl CoA.

Table 9.6. Nucleotides of Nicotinamide and Riboflavin.

Vitamin	*Nucleotide Complex*	*Components*
A. Nicotinamide	1. Nicotinamide-adenine dinucleotide or NAD^+	Nicotinamide- Ribose - Phosphate - Phosphate - Ribose - Adenine.
	2. Nicotinamide adenine dinucleotide phosphate or $NADP^+$	Nicotinamide - Ribose - Phosphate - Phosphate - Ribose - Adenine - Phosphate.
B. Riboflavin	1. Flavin mono-nucleotide or FMN.	Riboflavin - Phosphate.

	2. Flavin adenine dinucleotide or FAD.	Riboflavin - Phosphate- Phosphate - Ribose - Adenine.
C. Pantothenic Acid	Coenzyme A	Adenine - Ribose - Phosphate - Phosphate - Phosphate - Pantothenic Acid — Mercaptoethylamine

They may occur in the free state but are biologically active in contact with specific apoenzymes. The enzymes formed from them are dehydrogenases. The coenzymes oxidise different chemicals by removing hydrogen from them. The reduced coenzymes hand over their hydrogen to other chemicals.

$$NAD^+/NADP^+ + 2H \rightleftharpoons NADH/NADPH + H^+$$

$$FMN/FAD + 2H \rightleftharpoons FMNH_2/FADH_2$$

Functions of Nucleotides

1. They are **building blocks** of nucleic acids, ribonucleotides for RNAs while deoxyribonucleotides produce DNA.
2. **Cyclic AMP** (cAMP) functions as second chemical messenger in many hormone controlled chemical reactions.
3. **Cyclic GMP** (cGMP) is functional in Ca^{2+} or calmodulin mediated chemical reaction.
4. Higher nucleotides behave as **energy carriers**. ATP is known as energy currency of the cell since it contains high energy phosphate bonds which are built up when energy is available and broken down when energy is to be utilized.
5. Nucleotides produced by nicotinamide and riboflavin function as coeyzymes (NAD^+, $NADP^+$, FMN and FAD) of deydrogenases or oxidases.
6. CoA is produced by reaction between nucleotide and pantothenic acid.
7. UDP and ADP are involved in synthesis of polysaccharides while CDP and CTP are required in phospholipid synthesis.

NUCLEIC ACIDS

Nucleic acids are long chain macromolecules which are formed by end to end polymerisation of large number of repeated units called nucleotides. Nucleic acids show a wide variety of secondary structures. There are two types of nucleic acids— deoxyribonucleic acid or DNA and ribonucleic acid or RNA.

STRUCTURE

DNA or deoxyribose nucleic acid is a helically twisted double chain polydeoxyribonucleotide macromolecule which constitutes the genetic material of all organisms with the exception of riboviruses. In procaryotes it occurs in nucleoid and plasmids. This DNA is usually circular. In eucaryotes, most of the DNA is found in chromatin of nucleus. It is **linear**. Smaller quantities of DNA are found in mitochondria and plastids (organelle DNA). It may be circular or linear. Single-stranded DNA occurs as a genetic material in some viruses (*e.g.*, coliphage $\phi \times 174$). DNA is the largest **macromolecule** with a diameter of 2 nm (20Å)and having a length in millimetres. It is a long chain polymer of several hundred thousands of deoxyribonucleotides, *e.g.*, 4.7 million base pairs in *E.coli* and more than 3 billion base pairs in human being. A DNA molecule has two unbranched complementary strands. They are spirally coiled. The two spiral strands of DNA are collectively called **DNA duplex** (Fig. 9.21). The two strands are not coiled upon each other but double strand is coiled upon itself around a

common axis like a rope or spiral stair case with base pairs forming steps (rungs) while the back bones of the two strands form railings. Due to spiral twisting, the DNA duplex comes to have two types of alternate grooves, major and minor. One turn of 360° of the spiral has about 10 nucleotides on each strand of DNA. It occupies a distance of about 3.4 nm (34Å) *i.e.*, pitch of DNA is 34Å so that adjacent nucleotides or their bases are separated by a space of less than 0.34 nm (3.4 Å).

A deoxyribonucleotide of DNA is formed by cross-linking of three chemicals—phosphoric acid (H_3PO_4), deoxyribose sugar ($C_5H_{10}O_4$) and a nitrogen base. Four types of nitrogen bases occur in DNA. They belong to two groups, **purines** (9-membered double rings with nitrogen at 1,3,7 and 9 positions) and **pyrimidines** (six membered rings with nitrogen at 1 and 3 positions). DNA has two types of purines (**adenine** or A and **guanine** or G) and two types of pyrimidines (**cytosine** or C and **thymine** or T). Depending upon the type of nitrogen base, DNA has four kinds of deoxyribonucleotides —deoxy adenosine 5-monophosphate (d AMP), deoxy guaninosine 5–monophosphate (d GMP), deoxy thymidine 5-monophosphate (d TMP) and deoxy cytidine 5-monophosphate (d CMP).

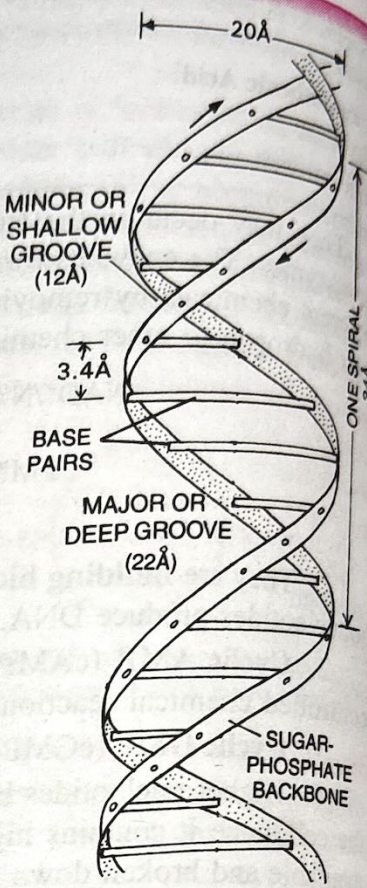


Fig. 9.21.Coiling in double helix or duplex of DNA.

The back bone of a DNA chain or strand is built up of alternate deoxyribose and phosphoric acid groups. The phosphate group is connected to carbon 5′ of the sugar residue of its own nucleotide and carbon 3′ of the sugar residue of the next nucleotide by phosphodiester bonds. –H of phosphate and –OH of sugar are eliminated as H_2O during each ester formation. At one end of DNA strand, last sugar has its 5-C free while at other end 3-C of first sugar is free. They are respectively called 5′ and 3′ ends. Phosphate group provides acidity to the nucleic acids because at least one of its side group is free to dissociate. Nitrogen bases lie at right angles to the longitudinal axis of DNA chains. They are attached to carbon atom 1′of the sugars by glycosidic bonds. Pyrimidine is attached to deoxyribose by its N-atom at 1′ position while a purine does so by N-atom at 9′ position.

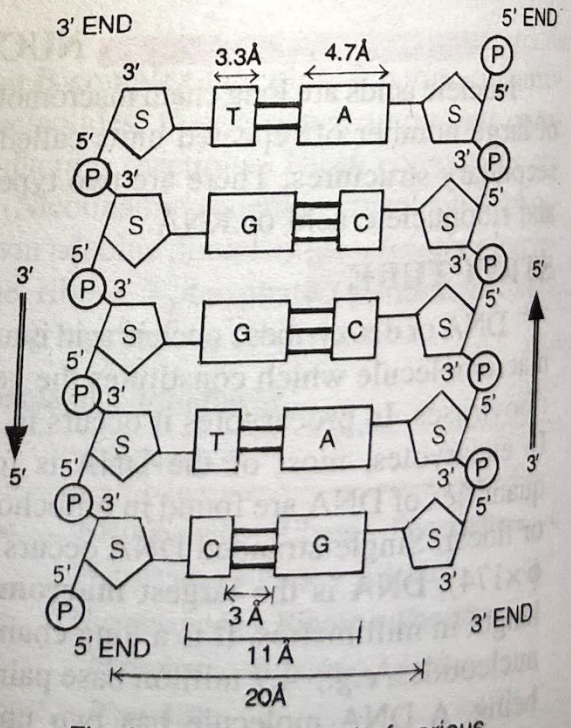


Fig. 9.22. Arrangement of various constituents of DNA duplex.

The two DNA chains are **antiparallel** that is, they run parallel but in opposite directions. In one chain the direction is 5′→3′ while in the opposite one it is 3′ →5′ (Fig. 9.22). The two chains are held together by hydrogen bonds between their bases. Adenine (A), a purine of one chain lies exactly opposite thymine (T), a pyramidine of the other chain. Similarly, cytosine (C, a pyramidine)

lies opposite guanine (G, a purine). This allows a sort of lock and key arrangement between large sized purine and small sized pyrimidine. It is strengthened by the appearance of hydrogen bonds between the two. Three hydrogen bonds occur between cytosine and guanine (C≡G) at positions 1–1′ , 2′ – 6′ and 6′ –2′ . There are two such hydrogen bonds between adenine and thymine (A=T) which are formed at positions 1′ –3′ and 6′ –4′ . Hydrogen bonds occur between hydrogen of one base and oxygen or nitrogen of the other base. Since specific and different nitrogen bases occur on the two DNA chains, the latter are **complementary**. Thus the sequence of say AAGCTCAG of one chain would have a complementary sequence of TTCGAGTC on the other chain. In other words, the two DNA chains are **not identical**. It is because of specific base pairing with a purine lying opposite a pyrimidine. This makes the two chains 2 nm thick. A purine-purine base pair will make it thicker while a pyrimidine–pyrimidine base pair will make it narrower than 2 nm. A larger sized purine, therefore, lies opposite the smaller-sized pyrimidine, A opposite T and C opposite G. This specific base pairing makes the two chains complementary.

Sense and Antisense Strands. Both the strands of DNA do not take part in controlling heredity and metabolism. Only one of them does so. The DNA strand which functions as template for RNA synthesis is known as **template strand, minus (–) strand** or **antisense strand.** Its complementary strand is named **nontemplate strand, plus (+) strand, sense** or **coding strand**. The latter name is given because by convention DNA genetic code is written according to its sequence.

(5′) G C A T T C G G C T A G T A A C (3′)
DNA Nontemplate, Sense (+) or Coding Strand
(3′) C G T A A G C C G A T C A T T G (5′)
DNA Template, Antisense, or Noncoding or (–) Strand
(5′) G C A U U C G G C U A G U A A C (3′)
RNA Transcript

RNA is transcribed on 3′ → 5′ (–) strand (template/antistrand) of DNA in 5 ←3 direction. The (+) strand of DNA is that coding strand which carries genetic information but is nontemplate.

The term antisense is also used in wider prospective for any sequence or strand of DNA (or RNA) which is complementary to mRNA.

Denaturation (= Melting). The hydrogen bonds between the nitrogen bases of complementary DNA strands can break due to high temperature, low or high *p*H. The phenomenon is called **denaturation** or **melting**. Since an A—T base pair has only two hydrogen bonds, the area rich in A—T base pairs can undergo easy denaturation. It is known as **low melting area**. The area rich in G—C base pairs is comparatively more stable becasue three hydrogen bonds connect the complementary nitrogen bases. DNA strands separated by melting can reassociate and form duplex. The phenomenon is called renaturation.

Palindromic and Repetitive DNA. DNA duplex possesses areas where sequence of nucleotides is the same but oppsite in the two strands, *e.g.*,

—T—T—A—A—C—G—T—T—A—A—
—A—A—T—T—G—C—A—A—T—T

These areas are called **palindromes** or palindromic regions. Regions conncected with transcription of ribosomal RNA are often palindromic. The exact significance of this sort of arrangement is not known.

Differences between Procaryotic DNA and Eucaryotic DNA

Procaryotic DNA	*Eucaryotic DNA*
1. DNA content is small, less than 0.1 pg.	1. DNA content is comparatively high, more than 1 pg.
2. DNA occurs freely inside the cytoplasm.	2. DNA does not lie freely in the cytoplasm.
3. There is nothing like nucleus.	3. Most of DNA lies inside the nucleus.
4. Organelle DNA is absent.	4. DNA is present in mitochondria and plastids.
5. It consists of a single molecule.	5. Eucaryotic DNA has several different molecules.
6. It is naked.	6. Nuclear DNA associated with histones with organelle DNA is naked.
7. It is generally circular.	7. Nuclear DNA is linear. Organelle DNA can be circular or linear.
8. Introns are rare.	8. A cistron often contains superfluous regions or introns.
9. Nonfunctional regions are fewer.	9. Nonfunctional DNA is quite abundant.
10. GC content is more than AT content.	10. AT content is generally more than that 4 CG.
11. Small DNA segments or plasmids may occur.	11. Plasmids are generally absent.
12. There are fewer genes.	12. Number of genes is more.

Functions of DNA

(1) DNA is genetic material which carries all the hereditary information coded in the arrangement of its nitrogen bases. (2) It has the property of replication (autocatalytic function) essential for passing genetic information from one cell to its daughters or from one generation to the next. (3) Crossing over produces recombinations. (4) Changes in sequence and number of nucleotides produce mutations. Mutations are the fountain head of all variations and formation of new species. (5) It gives rise to RNAs through transcription (**Heterocatalytic function**). (6) DNA controls the metabolic reactions of cells through RNAs and RNA-directed synthesis of proteins, enzymes and other biochemicals. (7) Differentiation of various body parts is due to differential functioning of specific parts of DNA. (8) Developmental stages occur in the life cycle of an organism by an internal clock of DNA functioning.

RIBONUCLEIC ACID (RNA)

RNA is a single strand or chain (ds RNA is reported in wound tumour virus, Rice Dwarf virus) which is formed by end to end polymerisation of a number of ribonucleotides or ribotides. The polynucleotide is characterised by the presence of ribose sugar and uracil instead of thymine. It is formed through **transcription** where one strand of DNA acts as a template. This is followed by **processing** to produce different types of RNAs. A strand of RNA contains 70–12000 ribonucleotides. At places it may appear partially double stranded due to folding of single strand. Four types of ribonucleotides occur in RNA. They are adenosine monophosphate, guanosine monophosphate, uridine monophosphate and cytidine monophosphate. A ribonucleotide is formed of ribose sugar, phosphoric acid and a nitrogen base. The four nitrogen bases present in RNA are adenine, guanine (purines), cytosine and uracil (pyrimidines). The union of nitrogen base is with carbon 1′ of ribose sugar by glycosidic bond through 3 N or 9 N region. Phosphate combines with carbon 5′ of its sugar and carbon 3′ of next sugar (phosphodiester bond) similar to one found in DNA strand. Nitrogen bases can be arranged in any sequence so that there is a high degree of specificity

as found in DNA. Purine and pyrimidine ratio is not unity in RNA. Sequence of nitrogen bases found on RNA strand is complementary to their sequence on DNA template. For example, a sequence of ATACTG of DNA template shall appear as UAUGAC over RNA. There are six types of RNAs— ribosomal, transfer, messenger, genomic, small nuclear and small cytoplasmic RNAs. Out of these the first three (rRNA, mRNA and tRNA) are major classes of RNAs that are involved in gene expression.

1. **Ribosomal RNA** (rRNA). It is the most abundant RNA (70-80% of total) which has 3–4 types. Some of its types (23S, 28S) are the longest of all RNAs. As the name indicates, rRNA is a constituent of ribosomes. Here it lies coiled in between and over the protein molecules. Depending upon their sedimentation coefficient, RNAs of eucaryotes are of four types— 28S, 18S, 5.8S and 5S. Procaryotic ribosomes have three types of RNAs— 23S, 16S and 5S. 28S, 5.8S and 5S (23S and 5S in procaryotes) occur in larger subunit of ribosome while 18S (16 S in procaryotes) is found in smaller subunit of ribosome. rRNA is transcribed in the form of a longer chain of 45S in eucaryotes and 30S in procaryotes. In eucaryotic transcript the arrangement in 5′ →3′ direction is 18S — 5.8S — 28S. Several methylations occur prior to removal of spacer RNA. Removal of spacer RNA breaks the transcript into 2–3 parts. 5S is often transcribed separately.

Functions. (i) rRNAs bind protein molecules and give rise to ribosomes. (ii) 3′ end of 18S rRNA (16S in procaryotes) has nucleotides complementary to those of cap region of mRNA. (iii) 5S rRNA and surrounding protein complex provide binding site for tRNA. rRNAs get associated with specific proteins to form ribosome subunits. 50S subunit of prokaryotic ribosome contains 23S rRNA, 5S rRNA and some 32 protein molecules. 30S subunit of prokaryotic ribosome has 16S rRNA and about 21 protein molecules. 60S subunit of eukaryotic ribosome contains 28S rRNA, 5S rRNA, 5.8S rRNA and about 50 protein molecules. 40S subunit of eukaryotic ribosome consists of 18S rRNA and some 33 protein molecules.

2. **Transfer RNA** (tRNA). It is also called soluble or sRNA. There are over 100 types of tRNAs. Transfer RNA constitutes about 15% of the total RNA. tRNA is the smallest RNA with 70–85 nucleotides and sedimentation coefficient of 4S. The nitrogen bases of several of its nucleotides get modified *e.g.,* pseudouridine (ψ), dihydrouridine (DHU), inosine (I). This causes coiling of the otherwise single-stranded tRNA into L-shaped form (three dimensional, Klug, 1974) or clover-like form (two dimensional, Holley, 1965). About half of the nucleotides are base paired to produce paired stems. Five regions are unpaired or single stranded— AA-binding site, T ψ C loop, DHU loop, extra arm and anticodon loop. (i) **Anticodon**. It is made up of three nitrogen bases for recognising and attaching to the codon of mRNA. (ii) **AA-Binding Site.** It lies at the 3′ end opposite the anticodon and has CCA—OH group. This CCA group is added after the transcription (5′ end bears G). Amino acid or AA-binding site and anticodon are the two **recognition sites** of tRNA. (iii) **T ψC Loop**. It contains pseudouridine. The loop is the site for attaching to ribosomes. (iv) **DHU Loop.** The loop contains dihydrouridine. It is binding site for aminoacyl synthetase enzyme. (v) **Extra Arm**. It is a variable site arm or loop which lies between T ψC loop and anticodon. The exact role of extra arm is not known.

Functions. (i) tRNA is **adapter molecule** which is meant for transferring amino acids to ribosomes for synthesis of polypeptides. There are different tRNAs for different amino acids. Some amino acids can be picked up by 2–6 tRNAs. tRNAs carry specific amino acids at particular points during polypeptide synthesis as per cidons of mRNA. Codons are recognised by anticodons of tRNAs. Specific amino acids are recognised by particular activating or aminoacyl synthetase enzymes. (ii) They hold peptidyl chains over the mRNAs.

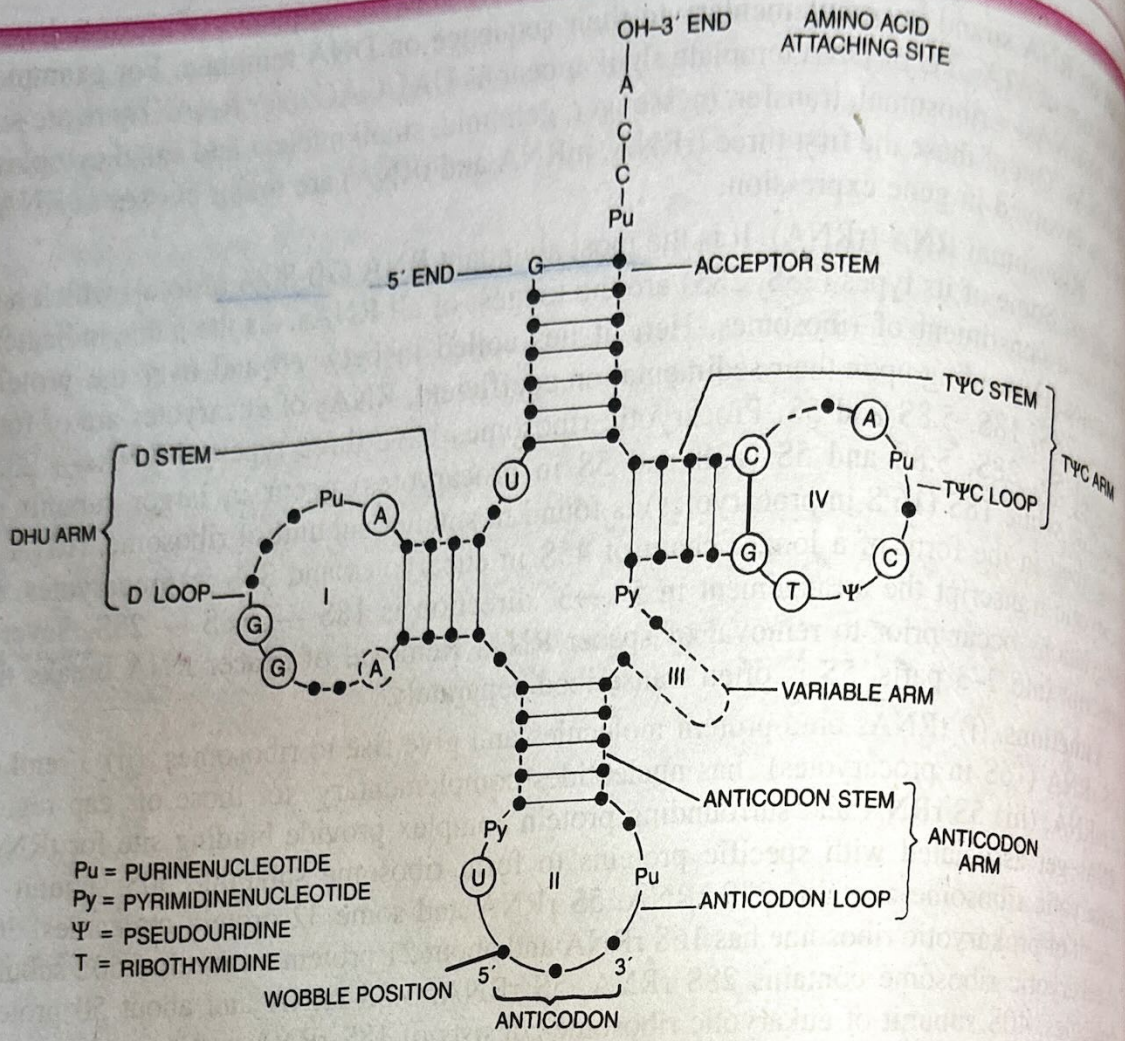


Fig. 9.23. Clover leaf model of tRNA.

3. **Messenger RNA** (mRNA). It is a long RNA which constitutes 2–5% of the total RNA content. It brings instructions from the DNA for the formation of particular type of polypeptide. The instructions are present in the base sequence of its nucleotides. It is called **genetic code**. Three adjacent nitrogen bases specify a particular amino acid. Formation of polypeptide occurs over the ribosome. mRNA gets attached to ribosome. tRNAs are induced to bring amino acids in a particular sequence according to the sequence of codons present over mRNA. mRNA has methylated region at the 5′ terminus. It functions as a **cap** for attachment with ribosome. Cap is followed by an **initiation codon** (AUG) either immediately or after a small noncoding region. Then there is coding region followed by **termination codon** (UAA, UAG, or UGA). There is then a small noncoding region and poly A area at the 3′ terminus (Fig. 9.24). An mRNA may specify only a single polypeptide or a number of them. The

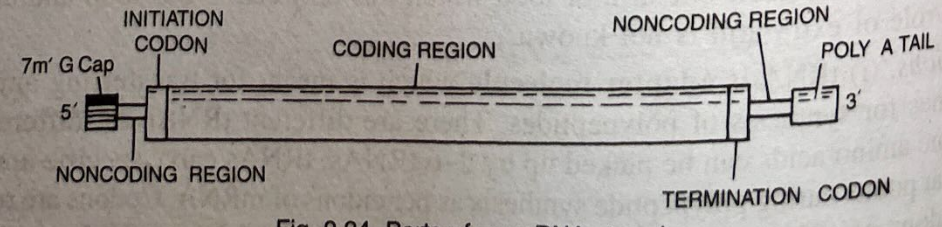


Fig. 9.24. Parts of an mRNA strand.

former is called **monocistronic** while the latter is known as **polycistronic**. Polycistronic mRNA is more common in prokaryotes. Eukaryotic mRNA is usually monocistronic. The life time of mRNA is also variable. In some lower forms it is from a few minutes to a few hours. On the other hand the mRNAs of higher forms seem to have a long life. It is several days in case of young red blood corpuscles which continue to form haemoglobin even when nucleus has degenerated.

Functions. (i) mRNA carries coded information for translation into polypeptide formation. (ii) Through reverse transcription it can form compact genes which are used in genetic engineering. The phenomenon also occurs in nature and has added certain genes in the genomes. (iii) It has a cap region for attachment to ribosome.

4. **Genetic RNA.** It is found in some viruses called riboviruses. Genetic RNA may be single stranded (*e.g.*, Tobacco Mosaic Virus or TMV) or double stranded (*e.g.*, Rheovirus). Genetic RNA acts as a hereditary material. It may, however, not replicate directly, but form DNA in the host cell to produce RNA of its own types.

Differences Between DNA and RNA

DNA	*RNA*
1. It usually occurs inside nucleus and some cell organelles.	1. Very little RNA occurs inside nucleus. Most of it is found in the cytoplasm.
2. DNA is the genetic material.	2. RNA is not the genetic material except in certain viruses, *e.g.*, *Reovirus*.
3. It is double stranded with the exception of some viruses (*e.g.*, phage f × 174).	3. RNA is single stranded with the exception of some viruses (*e.g.*, double stranded in *Reovirus*).
4. DNA contains over a million nucleotides.	4. Depending upon the type, RNA contains 70-12000 nucleotides.
5. Molecular weight ranges from 3-4 million in *Escherichia coli* to 263 million in chromosome 1 of human beings.	5. Molecular weight ranges from 25000-2,000,000.
6. It is Fuelgen positive.	6. RNA is Fuelgen negative.
7. It contains deoxyribose sugar.	7. It contains ribose sugar.
8. Nitrogen base thymine occurs in DNA alongwith three others — adenine, cytosine and guanine.	8. Thymine is replaced by uracil in RNA. The other three are similar — adenine, cytosine and guanine.
9. It replicates to form new DNA molecules.	9. It cannot normally replicate itself.
10. DNA transcribes genetic information to RNA.	10. RNA translates the transcribed message for forming polypeptides.
11. Its quantity is fixed for cell.	11. The quantity of RNA of a cell is variable.
12. DNA controls metabolism and genetics including variations.	12. It only controls metabolism under instructions from DNA.
13. Purine and pyrimidine bases are in equal number.	13. There is no proportionality between number of purines and pyrimidine bases.
14. It is long lived.	14. Some RNAs are very short lived while others have somewhat longer life.

Metabolism is the Total of All Chemical Reactions

Life is a never ending flow of energy and materials. The latter are required by all living cells for building and functioning of their living matter. Living cells also produce matter and energy which are passed out into the environment. **Metabolism** is the sum total of all chemical reactions occurring in an organism due to specific interactions amongst different types of molecules within the interior of cells. Despite vast differences occurring in structure

and functioning of cells in different organisms, metabolic reactions are unusually similar. There is a **turn-over** or rate at which new biomolecules are being formed from others while they are themselves being changed into others. Thousand of these transformations are going on simultaneously in cells, *e.g.*, removal of carbon dioxide from amino acids to form amines, removal of amino group from amino acid or nucleotide, hydrolysis of glycosidic bonds, etc. They occur in series called metabolic pathways. A **metabolic pathway** is a multistep interlinked series of enzyme catalysed biochemical reactions in which product of one reaction generally becomes substrate for the next. Metabolic pathways work in a linear fashion, often criss-crossing one another. There is no disruption or chaos as each and every metabolic pathway has a definite rate and direction that maintains the dynamic state of body constituents.

Enzymes Direct Metabolic Pathways. All chemical reactions occurring in the living systems are mediated through organic catalysts called enzymes. Like catalysts, enzymes remain unchanged at the end of the chemical reactions. They can, therefore, be used and re-used. Catalysts (*e.g.*, Platinum) are relatively unselective but enzymes are highly specific. There is a separate enzyme for every biochemical reaction, *i.e.*, there is no uncatalysed metabolic conversion in living systems. Even an otherwise physical process like dissolution of carbon dioxide in water is catalysed in living systems.

$$\underset{\text{Carbon dioxide}}{CO_2} + \underset{\text{Water}}{H_2O} \xrightarrow{\text{Carbonic anhydrase}} \underset{\text{Carbonic acid}}{H_2CO_3}$$

It is because rate of enzyme catalysed reaction is hundreds of times higher than the rate of uncatalysed reaction. In the absence of enzyme, nearly 200 molecules of carbon dioxide dissolve in water per hour to form carbonic acid. In the presence of enzyme carbonic anhydrase, some 600,000 molecules of carbonic acid are formed per second. This is an acceleration of about 10 million times.

During chemical reactions, older chemical bonds are broken and new chemical bonds are established, *e.g.*,

$$Ba(OH)_2 + H_2SO_4 \longrightarrow BaSO_4 + 2H_2O$$

It is an inorganic chemical reaction. Organic chemical reactions also occur similarly. Rate of chemical or a physical reaction is determined by the amount of product formed per unit time.

$$\text{Rate} = \frac{\delta P}{\delta t}$$

Rate of chemical reaction doubles or decreases by half for every 10°C rice or fall. It is called **general rule of thumb**.

As there are thousands of chemical reactions occurring in living cells, thousand of different types of enzymes develop inside the cells. Enzymes are generally globular proteins having one or more clefts over their surface. The clefts function as **active sites**. Active sites attract substrate molecules or reactants. An enzyme-substrate complex is formed. Chemical reaction occurs in this stage. It forms products. The products leave the active site which becomes free to attract more substrate molecules. The active site of each type of enzyme is specific for its substrate. This explains the specificity of enzymes. Sucrase will act only on sucrose and no other disaccharide.

$$\text{Substrate} \xrightarrow{\text{enzyme}} \text{Product}$$

$$\text{Sucrose} + H_2O \xrightarrow{\text{sucrase}} \text{Glucose} + \text{Fructose}$$

Types. Metabolism is of two kinds, catabolism and anabolism. **Anabolism** includes all the **"building up" reactions.** It is also called **constructive metabolism** since it involves the synthesis of complex substances from simpler ones, *e.g.*, synthesis of organic compounds from CO_2 and H_2O during photosynthesis, formation of starch from glucose, production of proteins from amino acids, formation of lipids from fatty acids and alcohols. Energy is stored (as potential energy) in the process.

$$6CO_2 + 12H_2O \xrightarrow[\text{enzymes}]{\text{chlorophyll, light energy,}} C_6H_{12}O_6 + 6H_2O + 6O_2\uparrow$$

$$n\,C_6H_{12}O_6 \xrightarrow{\text{enzymes, energy}} \underset{\textit{starch}}{(C_6H_{10}O_5)n} + nH_2O$$

Catabolism (= katabolism) constitutes **"breakdown reactions"**. It is also known as **destructive metabolism** because it involves breaking of complex substances into simpler ones. Potential energy present in the complex substances is converted into kinetic energy. Same of energy is trapped as chemical energy in **adenosine triphosphate** (ATP). The latter is also called energy currency of living systems. Respiration is an example of catabolism. It releases energy for performing different body activities.

$$C_6H_{12}O_6 + 6O_2 \xrightarrow{\text{enzymes}} 6CO_2 + 6H_2O + \text{energy}$$

Metabolism, therefore, involves changes in energy and materials. Bioenergetics is the scientific study of energy transformations in the living systems, *e.g.*, organisms, ecosystems.

Differences between Anabolism and Catabolism

Anabolism	*Catabolism*
1. It is the sum total of building up or constructive processes.	1. Catabolism is the sum total of breakdown or destructive processes.
2. Anabolism produces complex materials from simpler ones.	2. It forms simple substances from complex ones.
3. It stores energy.	3. It releases energy.
4. Kinetic energy is changed into potential energy.	4. Potential energy is changed into kinetic energy.
5. Anabolism is required for growth, maintenance and storage.	5. Catabolism is required for performing various activities of living beings.
6. Fewer types of precursors form diverse products (reactions diverge).	6. Many types of larger substances breakdown to form fewer types of simple molecules (reactions converge).

Biochemical Pathways are Tightly Regulated. Biochemical reactions do not occur singly. Neither they are unregulated. Unregulated anabolic and catabolic reactions occurring simultaneously can create chemical chaos as a substance systhesised in anabolism will be immediately broken down in catabolism. There can be excess synthesis or breakdown of a material resulting in unnecessary wastage of energy and materials. This does not happen. Actually cells have well separated biochemical pathways which are under tight control of regulatory systems which include control of enzyme synthesis, activation and inhibition of

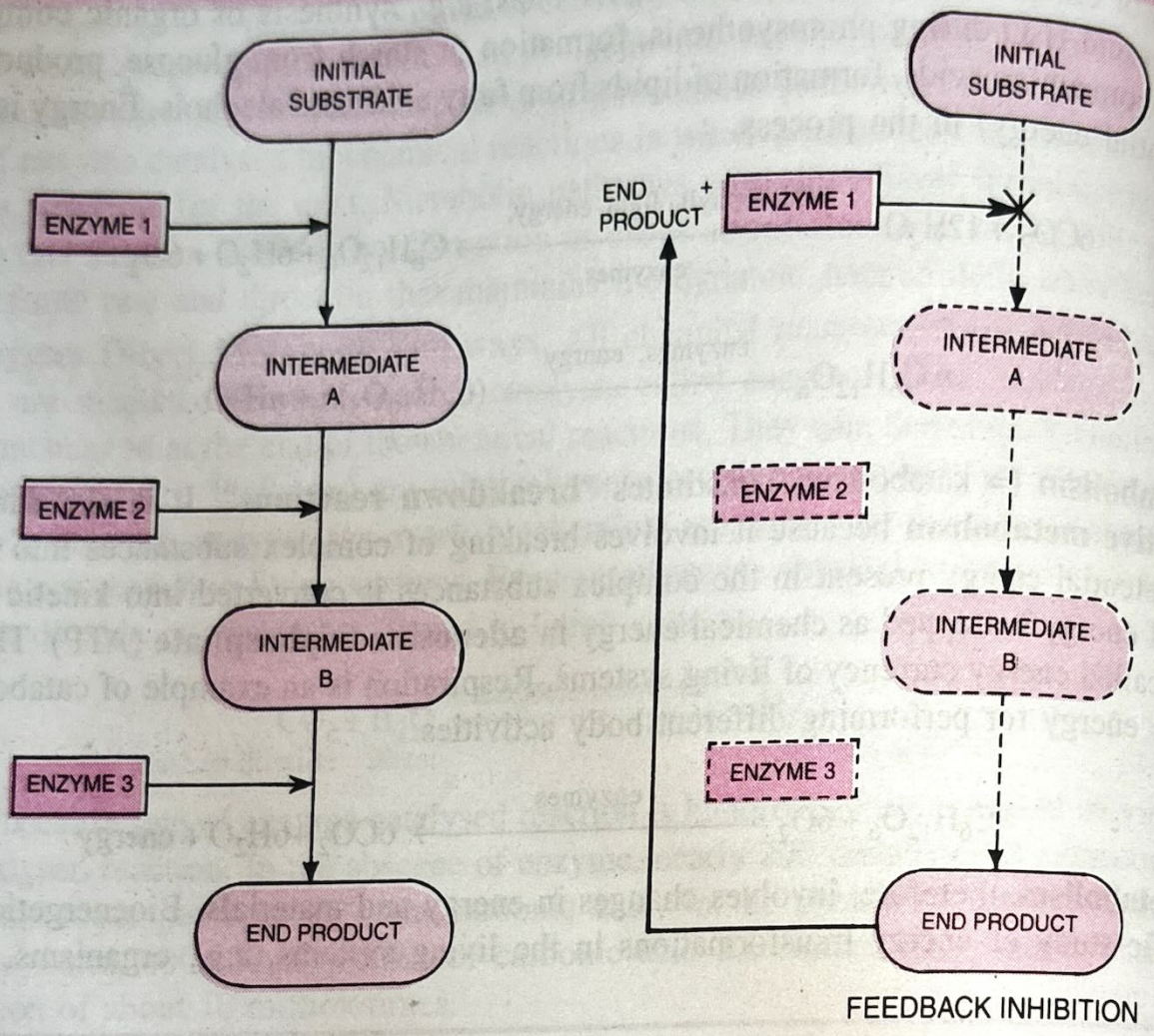


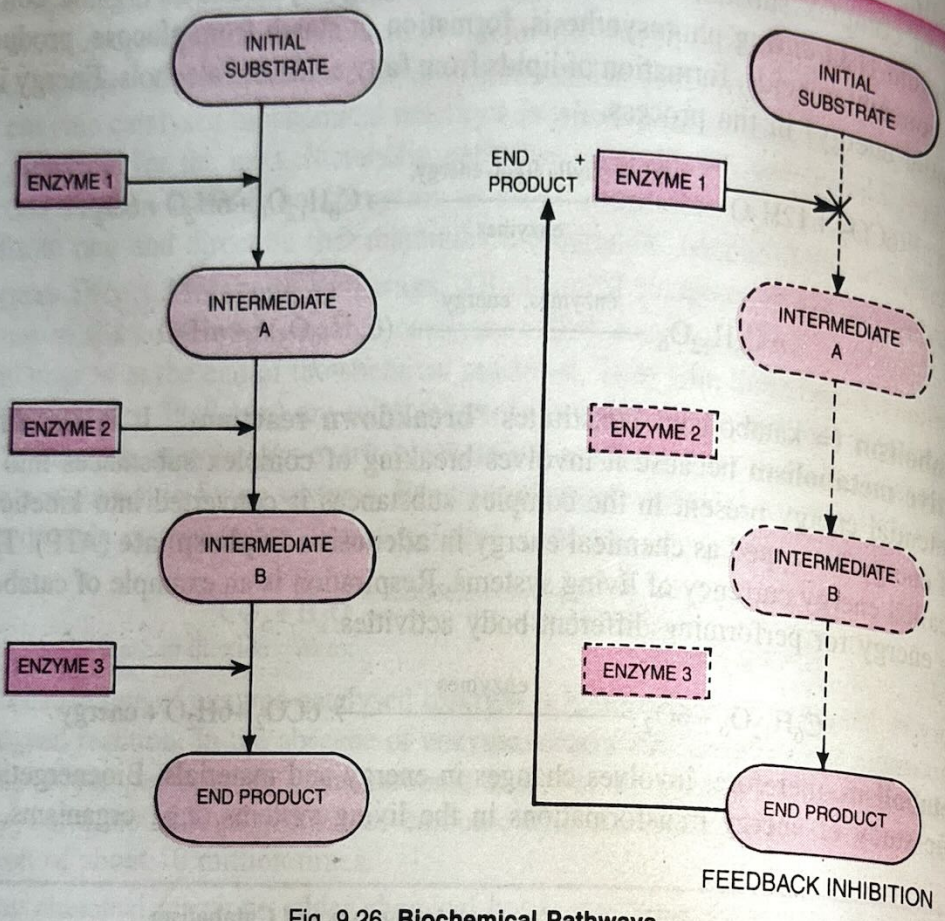
Fig. 9.26. **Biochemical Pathways.**

Further, a metabolic or biochemical pathway has a number of steps, each controlled by a separate enzyme. The pathway becomes operational only when the substrate of the first reaction and its active enzyme are available. The product of the first reaction generally becomes the substrate of the second reaction catalysed by a separate enzyme. The product of the second reaction becomes the substrate of the third reaction and so on till the final product is formed. The cells control the amount of final product as per their requirement by either controlling availability of the first substrate, first enzyme, utilisation of intermediate product or **feed back mechanism**. In feed back mechanism or inhibition, the excess final product functions as **allosteric inhibitor** of the first enzyme, *e.g.*, glucose 6-phosphate for enzyme hexokinase.

The Living State

Hundred and thounsands of metabolites or biomolecules occur in organisms in concentration characteristics of each of them, *e.g.*, glucose is 4.5–5.0 mM in blood, hormones in nanogram/ml. The living systems maintain this concentration of biomolecules because they are in metabolic flux, always remaining in **nonequilibrium steady state** where equilibrium is seldom achieved. No work can be carried out in equilibrium state. Therefore, living systems are regularly receiving an input of energy to prevent reaching an equilibrium and remain always in nonequilibrium steady state. Energy is obtained from metabolism. Metabo-

enzymes and feedback systems. Most enzymes have **allosteric sites** away from active sites. Presence of an activator over the allosteric site makes the active site functional while occurrence of an inhibitor over the allosteric site dysfunctions the active site.



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lism and living state are, therefore, complementary and synonymous. There can not be a living state without metabolism.

METABOLITES

Plants produce thousands types of chemicals. Some of the organic compounds like carbohydrates, fats, proteins, nucleic acids, chlorophylls, hemes are required for their basic metabolic processes and found throughout the plant kingdom. These organic compounds are called **primary metabolites** or biomolecules. These are produced in large quantities and can easily be extracted from the plants.

Table 9.7. Some Secondary Metabolites.

Pigments	Carotenoids, anthocyanins, etc.
Alkaloids	Morphine, Codeine, etc.
Terpenoides	Monoterpenes, Diterpenes, etc.
Essential oils	Lemon grass oil, etc.
Toxins	Abrin, Ricin
Lectins	Concanavalin A
Drugs	Vinblastin, curcumin, etc.
Polymeric substances	Rubber, gums, cellulose.

Many plants, fungi and microbes of certain genera and families synthesize a number of organic compounds which are not involved in primary metabolism (photosynthesis, respiration, protein and lipid metabolism) and seem to have no direct function in growth and development of plants. Such compounds are called **secondary metabolites** (secondary plant products or natural products) (Table 9.7). These compounds are accessary *rather than central* to the functioning of the plants in which they are found. These compounds are produced in small quantities and their extraction from the plant is difficult and expensive. They accumulate in small quantities only in specific parts of plants. These are derivatives of primary metabolites. By the cultivation of plant cells in culture media, secondary metabolites can be produced on large scale.

Differences in Primary and Secondary Metabolites

Primary Metabolites	*Secondary Metabolites*
1. These are biomolecules required for basic metabolic processes.	1. These are derivatives of primary metabolites which are not involved in bast.
2. These are produced in generous quantities and can easily be extracted from the plant.	2. These are produced in small quantities and their extraction from the plant is difficult.
3. These are found throughout the plant kingdom.	3. Particular secondary metabolites are found in one plant species or families and not all.
4. These are part of the basic molecular structure of the cell.	4. These are not part of the basic molecular structure of the cell.
5. They are highly useful to plant.	5. They have limited role in plant.
6. They are found from the start of plant life.	6. They are found at particular stages of development..

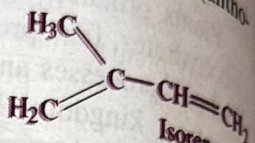
Role of Secondary Metabolites

(1) Some of them attract animals for pollination and seed dispersal. (2) They are used by the plants in their defense against herbivores and pathogens. (3) They act as agents of plant-plant competition. (4) They are used in making drugs, insecticides, flavours, pigments, scents, rubber, spices and other industrial materials like gums, resins for human welfare.

Types. These secondary metabolites are highly numerous in number, chemically diverse in nature and belong to three groups.

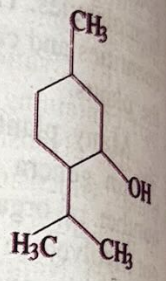
1. *Isoprenoids* or Terpenes, *e.g.*, rubber, steroids, essential oils, carotenoid pigments.
2. *Nitrogen* containing compounds, *e.g.*, alkaloids, glucosinolates, glycosides, nonprotein aminoacids.
3. *Phenolic compounds, e.g.*, lignin, tannins, coumarins, aflatoxins, flavonoids (anthocyanins).

1. **Isoprenoids** (Terpenes or Terpenoids). These are lipid like lipophilic compounds in plants and synthesized from acetyl coenzyme A through mevalonic pathway. It constitutes largest group of secondary metabolites. They are insoluble in water and are derived from the union of a 5 carbon unit called isoprene, *e.g.*,



Isorene

Monoterpenes (having two isoprene units and 10 carbon atoms)
Sesquiterpenes (having three isoprene units and 15 carbon atoms)
Diterpenes (having four isoprene units and 20 carbon atoms)
Triterpenes (having six isoprene units and 30 carbon atoms)
Tetraterpenes (having eight isoprene units 40 carbon atoms)
Polyterpenes (having large number of isoprene units)



Menthol

Role of Isoprenoids in Plants

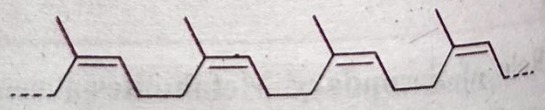
(a) **Monoterpenes** like pinene, myrcene are mainly found in resin ducts in leaves, twigs and trunks of conifers such as *pinus*. These are toxic to insects. They are also found in essential oils in secretory glands of some flowering plants like *menthol* (in peppermint oil obtained from mint plant), used in perfumery and flavouring food. Pyrethroids in flowers of *Chrysanthemum cinnerifolium* have strong insecticidal properties and used in making natural insecticides.

(b) **Sesquiterpene.** *Costunolide* in Sunflower has antifeedent properties to herbivores. *Gossypol* in cotton provides resistance against insects and other pathogens.

(c) **Triterpenes** and their derivatives such as *steroids* take part in making cell membranes and other defensive secondary products like terpenoid alkaloids and hormones, *e.g.*, *Limnoids* are bitter principles in citrus fruits; *Azadirachtin* from margosa (neem) tree is a strong deterrent to insect feeding, *saponins* have detergent properties; *yamogenin* and *diosgenin* from *Dioscorea* are used in making oral contraceptives.

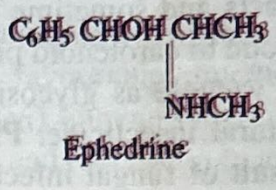
(d) **Polyterpenes** like rubber (latex), gutta, chicle are found in plants to provide defense against herbivores and to heal up wounds. Rubber is polyterpene made up of 1500 to 60000 isopentanyl units. It occurs in a milky emulsion called *Latex*. Latex has rubber, water, resins, terpenes, proteins and sugars. It is produced in *phloem* and accumulates in laticifers. These are used in industries to make rubber items, guttapercha, chewing gums.

(e) Some terpenoids have a primary role in growth and development of plants, for example gibberellins (a diterpene), ABA (a sesquiterpene), sterols (triterpenes), carotenoids (tetraterpenes), phytol tail of chlorophylls is diterpene. Carotenoids are accessary pigments to protect chlorophyll from photooxidation.

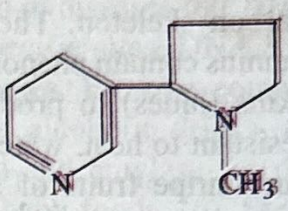

Rubber

2. **Nitrogen containing secondary metabolites**. These are synthesized in plants from aminoacids. These are of three types; alkaloids, cyanogenic glycosides and nonprotein aminoacids.

(a) **Alkaloids** are nitrogen containing heterocyclic compounds. They are alkali like, basic in nature mostly nonvolatile, solid, colourless, crystalline, *bitter* in taste, insoluble in water and used widely in pharma industry, *e.g.*, ephedrine (from *Ephedra* plant and used in cough), *caffeine* (from coffee and tea) theine (from tea) *nicotine* (tobacco plant), quinine (from cinchona plant), *colchicine* (from *Colchicum*), Cannabidiol (from hemp or Bhang plant), Vinblastin (from *Catheranthus*). They provide protection against predators, act as nitrogen reserve, growth regulator (inhibit seed germination), and maintain ionic balance.



Ephedrine

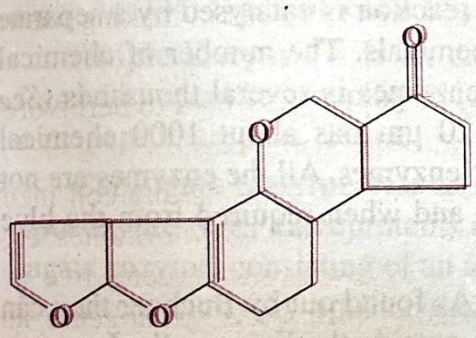


Nicotine

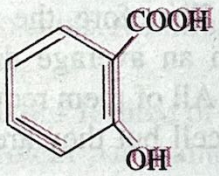
(b) **Cyanogenic glycosides** release poisonous HCN when plant part containing them is crushed and they come in contact with enzyme glycosidase and lyase. Thus they play a defensive role in plants. Glucosinolates in mustard oil contain N and S both and responsible for pungent taste of mustard, cabbage, cauliflower and other crucifers.

(c) **Nonprotein amino** acids are other than 20 protein amino acids. They occur free in plant cells. They are protective against herbivores, *e.g.*, ornithine, albizzine, canavanine.

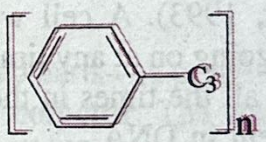
3. **Phenolic Compounds** (Plant Phenolics). These secondary metabolites are aromatic compounds and synthesized in plants either from acetyl CoA or erythrose phosphate and phosphoenol pyruvate. They have one or more phenolic residue, *e.g.*, coumarins, lignins, tannins, flavonoids, ferulic acid, salicylic acid etc. They act as chemical deterrents against herbivores and pathogens. Some phenolics help in attracting pollinators and fruit dispersers



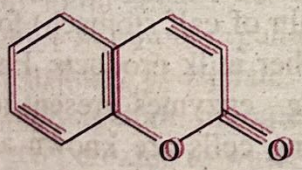
Aflatoxin



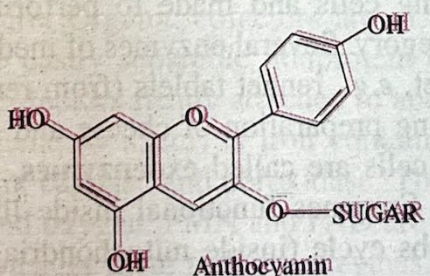
Salicylic acid



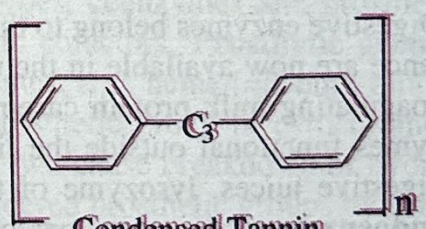
Lignin



Coumarin



Anthocyanin



Condensed Tannin

and some (*e.g.*, ferulic acid, caffeic acid) have allelopathic (ammensalism) activity. They protect cell from UV rays. Coumarins are antimicrobial, feeding deterrents and germination inhibitors. *Aflatoxin* is a coumarin produced by *Aspergillus flavus*. It is a mycotoxin and prevents RNA transcription. **Lignin** is a highly complex, branched polymer of phenolic compounds with $(C_6—C_3)_n$ carbon skeleton. It has three phenyl propanoid alcohols and provide mechanical strength to the plants. Flavonoids (like anthocyanins, flavones, isoflavonoids) are 15 carbon phenolic compounds with $C_6—C_3—C_6$ carbon skeleton. They help in defense and pigmentation. The brilliant pink, purple, blue, scarlet, yellow colours of petals, fruits,

bracts and sometime leaves and roots are due to anthocyanins (but colour of tomato fruits is due to carotenoid pigments). These are water soluble pigments, found dissolved in cell sap of vacuole as glycosides. The **rotenone** obtained from roots of *Derris* is widely used as a natural insecticide. **Phytoalexins** are antimicrobial isoflavonoids, produced in plants as a result of fungal infection.

Tannins are phenolic polymer of 15-Carbon phenolic compound with $C_6—C_3—C_6$ carbon skeleton. They are of two types, condensed and hydrolysable. The hydrolysable tannins contain phenolic acids like gallic acid and simple sugar. Tannins are used to tan animal skins (hides) to produce leather. They bind with collogen protein of skin to make them resistant to heat, water and microbes. They are also used to make inks. Barks, leaves, wood and unripe fruits of some plants are good sources of tannins. In plants, tannins provide protection against microbial attack.

ENZYMES

Enzymes are commonly proteinaceous substances which are capable of catalysing chemical reactions of biological origin without themselves undergoing any change. Therefore, they are called **biocatalysts**. Enzymes are synthesised by living cells. The term 'enzyme' was coined by Kuhne (1878) for catalytically active substances previously called **ferments**. Enzymes were actually found out by Buchner (1897) with the accidental discovery that fermentation of sugar is not only caused by living yeast cells but also yeast extract. The extract obviously possessed biocatalysts required for the process (Gk. *en.*– in, *zyme*– yeast). Buchner (1903) also isolated the first enzyme. He was awarded Nobel Prize in the same year, 1903. There are numerous enzymes as every biochemical reaction is catalysed by a separate enzyme. It is estimated that a cell contains over 5000 chemicals. The number of chemical reactions is many times more. Therefore, the number of enzymes is several thousands (*See* Lehninger, 1993). A cell with an average diameter of 20 μm has about 1000 chemical reactions going on at any time. All of them require specific enzymes. All the enzymes are not present at all the times in the cell but they are formed as and when required from the blue print present in DNA.

Enzymes are mainly functional inside the living cells. As found out by Buchner they can be extracted from the cells and made to catalyse reactions outside the living cells. In nature some enzymes are secreted by living cells and made to perform extracellular catalysis. Digestive enzymes belong to this category. Several enzymes of medical and chemical importance are now available in the market, *e.g.*, rennet tablets (from rennin of calf stomach) for coagulating milk protein casein during preparation of cheese and other milk products. Enzymes functional outside the living cells are called **exoenzymes**, *e.g.*, enzymes present in digestive juices, lysozyme of tears. Enzymes functional inside living cells are known as **endoenzymes**, *e.g.*, enzymes of Krebs cycle (inside mitochondria), enzymes of glycolysis (inside cytoplasm).

The biochemical which is acted upon by an enzyme is known as **substrate**. In case two biochemicals are involved in a reaction, the same are called **reactants**. The chemicals formed after the completion of a reaction are termed as **products**. The final products are also called **end products**. Part of enzyme that takes part in catalysing biochemical reaction is called **active site**.

Biological Importance. (i) Thousands of chemical reactions are taking place in the body of a living organism. All of them are mediated by enzymes. (ii) Enzymes are specialised catalysts that operate at biological temperatures. (iii) Enzyme mediated reactions do not

require harsh treatment. (iv) They are *p*H specific so that reactions requiring different *p*H operate in different parts of the body. (v) As they operate under favourable conditions, enzymes force the organisms to live under favourable environment. (vi) Enzymes are highly regulated. Their formation is controlled by separate genes. Activation and repression of genes allow certain enzymes to be functional or nonfunctional in cells.

Economic Importance. (1). **ELISA.** It is enzyme based detection of diseases like AIDS. (2). **Endonucleases.** They are enzymes used in breaking DNA at specific sites. DNA fragments are employed in genetic engineering. (3). **Alcoholic Beverages.** Enzyme complex zymase obtained from yeast is used in brewing or fermentation of alcoholic drinks. (4). **Detergents.** They contain protease for brighter washing of clothes and amylase for dish washing. (5). **Baby Foods.** Trypsin is added to partially pre-digest baby foods. (6). **Streptokinase.** The enzyme is used in clearing blood clots inside blood vessels. (7). **Digestive Aids.** Diastase and other enzymes are used regularly by patients with deficient digestive juices. (8). **Cheese.** Rennet or rennin tablets are used for preparation of cheese. Lactase and lipase are employed to provide proper consistency and flavour to cheese. (9). **Pectinase.** It is used for clearing fruit juices, retting of fibres and preparation of green coffee. (10). **Protease.** The enzyme is employed for chill-proofing of beverages, degumming of silk, cleaning of hides, softening of bread and meat.

Chemical Nature of Enzymes

All enzymes are globular proteins (Sumner, 1926) with the exception of recently discovered RNA enzymes. Some enzymes may additionally contain a nonprotein group. Accordingly there are two types of enzymes, simple and conjugate.

Simple Enzyme. It is an enzyme which is wholly made up of protein. Active site is formed by specific grouping of its own amino acids. Additional substance or group is absent, *e.g.*, pepsin, trypsin, urease.

Conjugate Enzyme. It is an enzyme which is formed of two parts— a protein part called **apoenzyme** (*e.g.*, flavoprotein) and a nonprotein part named **cofactor**. The complete conjugate enzyme, consisting of an apoenzyme and a cofactor, is called **holoenzyme**. Active site is formed jointly by apoenzyme and cofactor.

Cofactor is small, heat stable and dialysable part of conjugate enzyme. It may be inorganic or organic in nature. Organic cofactors are of two types, coenzymes and prosthetic groups. **Coenzymes** are easily separable nonprotein organic cofactors. **Prosthetic groups** are nonprotein organic cofactors firmly attached to apoenzymes, *e.g.*, heme (=haem), biotin, pyridoxal phosphate. Heme (= haem) is iron containing prosthetic group in cytochromes, haemoglobin, myoglobin, catalase and peroxidase. The last two cause breakdown of hydrogen peroxide to water and oxygen. FMN and FAD are considered prosthetic groups by some workers while others consider them to be coenzymes. Both coenzyme and prosthetic group take part in group transfer reactions. Prosthetic group requires a single apoenzyme for picking up the group and transferring the same. Coenzyme requires two apoenzymes, one for picking up the group and the second for transferring the group, *e.g.*, NAD^+, $NADP^+$, CoA. Coenzyme has three important functions (a) Coenzyme is essential for bringing the substrate in contact with the enzyme. (b) It picks up a product of the reaction, *e.g.*, hydrogen in case of NAD^+ (nicotinamide adenine dinucleotide) or $NADP^+$. (c) The product picked up by a coenzyme is transferred to another reactant.

Certain workers use the term **cofactor** for any loosely bound nonprotein group. The organic cofactor is called coenzyme. They use the term prosthetic group similarly for both inorganic and organic group attached firmly to apoenzyme.

Differences between Apoenzyme and Coenzyme

Apoenzyme	*Coenzyme*
1. It is protein part of the holoenzyme or conjugate enzyme.	1. Coenzyme is the nonprotein organic group which gets attached to the apoenzyme to form holoenzyme or conjugate enzyme.
2. It is large in size.	2. It is small in size.
3. An apoenzyme is specific for an enzyme.	3. A coenzyme can function as a cofactor for a number of enzymes carrying out that particular type of reaction.
4. It takes part in only catalytic activity of the enzyme.	4. It helps in removing a product of chemical reaction besides bringing contact between the substrate and the enzyme.
5. Apoenzyme does not help in group transfer.	5. Coenzyme takes part in group transfer.
6. Apoenzyme is thermolabile.	6. Coenzyme is heat stable.

Differences between Prosthetic Group and Coenzyme

Prosthetic Group	*Coenzyme (Cofactor)*
1. It is a nonprotein group attached firmly to an apoenzyme.	1. It is a nonprotein group attached loosely to an apoenzyme.
2. It requires a single apoenzyme for picking up and transferring a group. **Example :** Heme	2. It requires different apoenzymes for picking up and transferring a group. **Example :** NAD^+, $NADP^+$.

Most of the coenzymes are made of water soluble vitamins, B and C, *e.g.*, thiamine, riboflavin, nicotinamide, pyridoxine. **Inorganic cofactors** include ions of a variety of minerals *e.g.*, calcium, iron, copper, zinc, magnesium, manganese, potassium, nickel, molybdenum, selenium, cobalt. They usually function as **activators** by forming one or more **coordination bonds** with both the substrate and active site of enzyme. Fe^{2+} is cofactor for catalase. Chloride ion stimulates activity of salivary amylase. Zinc is required for carboxypeptidase NAD^+ and $NADP^+$ activity.

Active Site or Active Spot

The whole of enzyme molecule is not active in catalysing a chemical reaction. Only a small portion of it is active. It is called **active site** or **active spot**. An enzyme may have one to several active sites. *An active site or spot is an area of the enzyme which is capable of attracting and holding particular substrate molecules by its specific charge, size and shape so as to allow the chemical change.* It fails to recognise other molecules. Active site consists

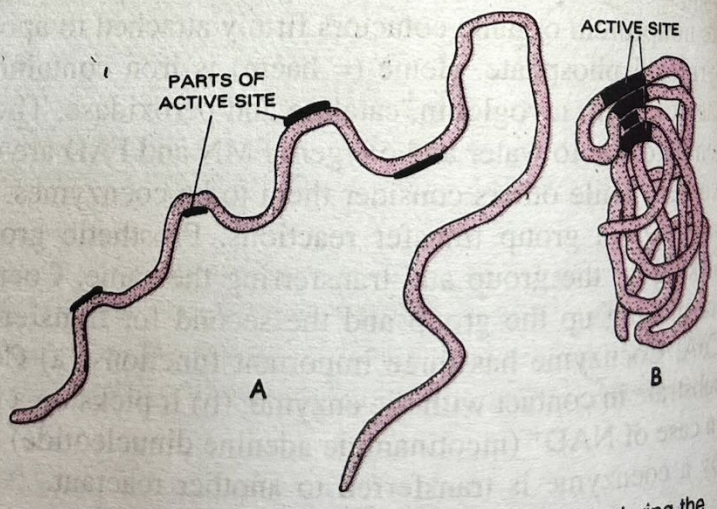


Fig. 9.27. Grouping of amino acids of a polypetide during the formation of tertiary structure to produce an active site.

of a few amino acids and their side groups which are brought together in a particular fashion due to secondary and tertiary folding of a protein molecule (Fig. 9.27) and its association with the cofactor, if any. For example, the active site for aldolase is glycine-histidine-alanine while that of pyruvic oxidase is aspartic acid-cysteine-alanine. The remaining amino acids help maintain the shape of the enzyme molecule.

Nomenclature

All enzyme names should end in suffix –ase. Exceptions are some old names, *e.g.*, ptyalin, pepsin, trypsin. Some old names indicate the source but not the action, *e.g.*, papain from Papaya, bromelain from Pineapple of family Bromeliaceae. In modern system enzyme names are given after (i) **Substrate acted upon**, *e.g.*, sucrase (after sucrose), lipase, proteinase, nuclease, peptidases, maltase (ii) **Chemical reaction**, *e.g.*, dehydrogenase, oxidase, carboxylase, decarboxylase, etc. The second category of names are group names. They are often qualified by the addition of the name of substrate, *e.g.*, succinic dehydrogenase, isocitric dehydrogenase, glutamate-pyruvate transaminase, DNA polymerase. Thus DNA polymerase catalyses synthesis of DNA segments through polymerisation of deoxyribonucleotides. Similarly glutamate-pyruvate transaminase transfers amino group ($—NH_2$) from glutamate to pyruvate.

Classification

In older times enzymes were classified into two broad categories. (i) **Hydrolysing.** Catalysing hydrolysis of larger molecules into smaller ones, *e.g.*, carbohydrates or amylases, proteases, lipases, esterases, phosphorylases, amidases. Digestive enzymes are hydrolysing in nature. They are often grouped into three types— proteolytic, amylolytic and lipolytic. (ii) **Desmolysing.** Catalysing reactions other than hyrolysis, *e.g.*, aldolases, dehydrogenases, oxidases, peroxidases, catalases, carboxylases, etc.

The modern system of enzyme classification was introduced by International Union of Biochemistry (IUB) in 1961. It groups enzymes into the following six categories.

1. **Oxidoreductases**. They take part in oxidation and reduction reactions or transfer of electrons.

$$S \text{ reduced} + S' \text{ oxidised} \xrightarrow{\text{oxido reductase}} S \text{ oxidised} + S' \text{ reduced}$$

Oxidoreductases are of three types— oxidases, dehydrogenases and reductases, *e.g.*, cytochrome oxidase (oxidises cytochrome), succinate dehydrogenase, nitrate reductase.

$$2AH_2 + O_2 \xrightarrow{\text{oxidase}} 2A + 2H_2O$$

$$AH_2 \xrightarrow{\text{dehydrogenase}} A + 2(H)$$

$$A + 2(H) \xrightarrow{\text{reductase}} AH_2$$

2. **Transferases**. They transfer a group from one molecule to another *e.g.*, glutamate-pyruvate transaminase (transfers amino group from glutamate to pyruvate during synthesis of alanine). The chemical group transfer does not occur in the free state.

$$S - G + S' \xrightarrow{\text{transferase}} S + S' - G$$

$$\underset{\text{(amino acid)}}{\text{Glutamic Acid}} + \underset{\text{(organic acid)}}{\text{Oxaloacetic Acid}} \xrightarrow{\text{transferase}} \underset{\text{(organic acid)}}{\alpha\text{-ketoglutaric Acid}} + \underset{\text{(amino acid)}}{\text{Aspartic Acid}}$$

3. **Hydrolases**. They catalyse hydrolysis of bonds like ester, ether, peptide, glycosidic, C—C, C—halide, P—N, etc. which are formed by dehydration condensation. Hydrolases break up large molecules into smaller ones with the help of hydrogen and hydroxyl groups of water molecules. The phenomenon is called hydrolysis. Digestive enzymes belong to this group, *e.g.*, amylase (hydrolysis of starch), sucrase, lactase.

$$\underset{\text{maltose}}{C_{12}H_{22}O_{11}} + H_2O \xrightarrow{\text{maltase}} 2\,\underset{\text{glucose}}{C_6H_{12}O_6}$$

4. **Lyases**. The enzymes cause cleavage, removal of groups without hydrolysis, addition of groups to double bonds or removal of a group producing double bond, *e.g.*, histidine decarboxylase (breaks histidine to histamine and CO_2), aldolase (fructose-1, 6-diphosphate to dihydroxy acetone phosphate and glyceraldehyde phosphate).

$$\begin{array}{cc} X & Y \\ | & | \\ C & -C \end{array} \xrightarrow{\text{lyase}} X-Y + C=C$$

$$\text{Fructose 1, 6-diphosphate} \xrightarrow{\text{aldolase}} \text{Dihydroxy acetone phosphate} + \text{Glyceraldehyde phosphate.}$$

5. **Isomerases**. The enzymes cause rearrangement of molecular structure to effect isomeric changes. They are of three types, isomerases (aldose to ketose group or *vice-versa* like glucose 6-phosphate to fructose 6-phosphate), epimerases (change in position of one constituent or carbon group like xylulose phosphate to ribulose phosphate) and mutases (shifting the position of side group like glucose-6-phosphate to glucose-1- phosphate).

$$\text{Glucose 6-phosphate} \xrightarrow{\text{isomerase}} \text{Fructose 6-phosphate}$$

$$\text{Glucose 6-phosphate} \xrightarrow{\text{mutase}} \text{Glucose 1-phosphate}$$

$$\text{Xylulose 5-phosphate} \xrightarrow{\text{epimerase}} \text{Ribulose 5-phosphate}$$

6. **Ligases** (Synthetases). The enzymes catalyse bonding of two chemicals with the help of energy obtained from ATP resulting in formation of such bonds as C—O, C—S, C—N and P—O, *e.g.*, pyruvate carboxylase. It combines pyruvic acid with CO_2 to produce oxaloacetic acid.

$$\text{Pyruvic acid} + CO_2 + \text{ATP} + H_2O \xrightleftharpoons{\text{Pyruvate Carboxylase}} \text{Oxaloacetic acid} + \text{ADP} + \text{Pi}$$

Characteristics (Properties of Enzymes)

1. **Protein Nature**. Enzymes are generally globular proteins. They may have additional inorganic or organic substances for their activity. However, two types of RNA enzymes are known, **ribozyme** (Cech *et al*, 1981, for removing introns) and **ribonuclease-P** (Altman *et al*, 1983, for separating tRNAs from hnRNA). Peptidyl transferase has also been found to be part of rRNA by Noller *et al* (1992).

2. **Molecular Weight**. Being proteinaceous, the enzymes are giant molecules with a molecular weight of 6000 (bacterial ferredoxin) to 4,600,000 (pyruvate dehydrogenase complex).

3. **Colloidal Nature.** They are hydrophilic and form hydrosol in the free state.

4. **Chemical Reaction**. Enzymes do not start a chemical reaction but increase the rate

of chemical reaction. They do not change the equilibrium but bring about equilibrium very soon.

5. **Efficiency**. The number of substrate molecules changed per minute by a molecule or enzyme is called **turn over number** (k_{cat}). The higher the turn-over number, the more efficient an enzyme is. It depends upon the number of active points present over an enzyme, precise collisions between reactants and the rate of removal of end products. The optimum turn-over number for enzyme carbonic anhydrase (enzyme present in RBCs) is 36 million, catalase 5 million, enzyme sucrase or invertase 10,000 and flavoprotein 50. Enzyme efficiency is usually much more than that of inorganic catalysts. For exampe, the rate of enzyme mediated hydrolysis of urea is 10^{14} times higher than the rate of its acid hydrolysis carried out at 40°C higher temperature. Similarly, the rate of CO_2 hydration is 10 million times faster in the presence of enzyme carbonic anhydrase than in its absence.

6. **Unchanged Form**. Enzymes are in no way transformed or used up in the chemical reaction but come out unchanged at the end of reaction.

7. **Reversibility**. Theoretically, all enzyme controlled reactions are reversible. Reversibility is, however, dependent upon energy requirements, availability of reactants, concentration of end products and *p*H.

8. **Enzyme Specificity**. Enzymes are highly specific in their action. For example, enzyme maltase acts on sugar maltose but not on lactose or sucrose. Different enzymes may act on the same substrate but give rise to different products. For example, raffinose gives rise to melibiose and fructose in the presence of enzyme sucrase while in the presence of enzyme melibiase it produces lactose and sucrose. Similarly an enzyme may act on different substrates, *e.g.*, sucrase can act on both sucrose and raffinose producing different end products.

Experiment 1. Prove that enzymes are specific in their activity.

Apparatus. Four test tubes, one percent starch solution, one percent sucrose solution, saliva, Bennedict's or Fehling's solution, spirit lamp.

Working. Take four test tubes. Pour 1 ml of 1% starch solution in the first, 1 ml of 1% sucrose solution in the second, 1 ml each of 1% starch solution and saliva in the third, and 1 ml each of 1% sucrose solution and saliva in the fourth. Keep all the test tubes undisturbed for one hour at room temperature if it is summer and in warm water (about 38°C) if winter. Afterwards pour 3 ml of Bennedict's or Fehling's solution (having cupric ions) in each of the test tube. Heat the test tube to boiling for about 2 minutes and observe. There is no change of colour in first and second test tubes. The fourth test tube also does not show any change in colour. However, in the third test tube, the blue colour of Bennedict's or Fehling's solution gets changed to yellowish or reddish precipitate.

Result. The change of colour from blue to yellowish or reddish precipitate is caused by conversion of cupric ions of Bennedict's or Fehling's solution into cuprous oxide. This occurs in the presence of reducing sugars. Both sucrose and starch are non-reducing in nature as is found by the absence of colour change in test tube one and two. In test tube four having sucrose and saliva there is no enzyme activity since colour change is absent. However, test tube three having starch and saliva (containing enzyme salivary amylase) reducing sugars are produced as is clear from change of colour. Therefore, the enzyme, salivary amylase, present in saliva catalyses hydrolysis of starch but not sucrose which is a common disaccharide.

Precautions. (i) Test tubes must be thoroughly washed and dried. (ii) The water in which the solutions are to be kept must not be very hot. (iii) In winter warm water must be taken. (iv) Before the collection of saliva, mouth must be washed thoroughly with distilled water. (v) Swill the rinsed mouth with 7-10 cc of distilled water for 1minute and then collect the same, as water containing saliva. (vi) While heating the solution should not be allowed to bump violently.

9. **Heat Sensitivity**. All enzymes are heat sensitive or thermolabile. Most enzymes operate optimally between 25°–35°C. They become inactive at freezing temperatures and denatured at 50°–55°C. However, thermal algae and bacteria are an exception. Their enzymes

remain functional even at 80°C. Enzymes of seeds and spores are also not denatured at 60°–70°C.

10. **Protein Poisons.** Being made of proteins, enzymes are inactivated or denatured by all those substances and forces which destroy protein structure, *e.g.*, heavy metals, high energy radiations.

11. ***p*H.** Each enzyme functions at a particular *p*H (Fig. 9.28.), *e.g.*, pepsin (2 *p*H), sucrase (4.5 *p*H), salivary amylase (6.8 *p*H), trypsin (8.5 *p*H). A change in *p*H makes the enzymes ineffective.

Specificity of *p*H for enzyme activity is useful in regulating enzymes, *e.g.*, salivary amylase stops its activity in stomach where hydrochloric acid is secreted. The same acid activates another enzyme pepsin from its precursor called pepsinogen. Pepsinogen can also be changed into pepsin by catalytic activity of the latter.

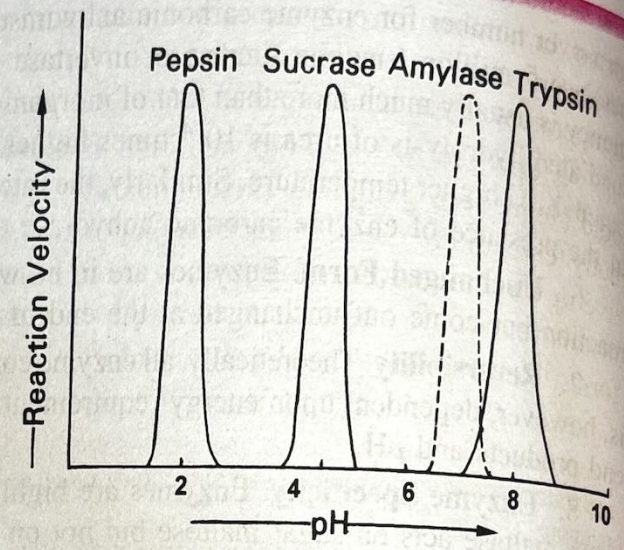
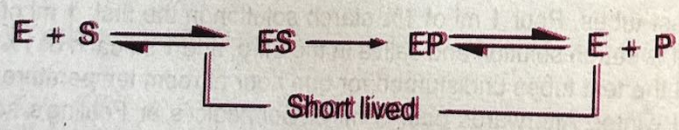


Fig. 9.28. Relation between *p*H and activity of four enzymes.

12. **Enzyme–Substrate Complex.** The active sites of enzymes have a specific conformation for attracting and holding substrate. It usually possesses a crevice or pocket where the substrate fits in a complementary fashion. The two join to form a complex known as enzyme–substrate complex (ES). The complexed state is short lived. The substrate is changed into products. The products remain complexed with the active site of the enzyme for a brief period. They soon separate and the active site is freed to perform another catalytic act.



The greater the affinity of the enzyme for a substrate, the higher is the catalytic activity.

13. **Chain Reactions.** Biochemical reactions are not isolated. A number of them occur in quick succession. A team of enzymes work one after the other to accomplish such multistep reactions, *e.g.*, five enzymes for conversion of threonine to isoleucine.

Factors Influencing Enzyme Activity

1. **Temperature.** An enzyme is active within a narrow range of temperature. The temperature at which an enzyme shows its highest activity is called **optimum temperature** (Fig. 9.29). It generally corresponds to the body temperature of warm blooded animals, *e.g.*, 37°C in human beings. Enzyme activity decreases above

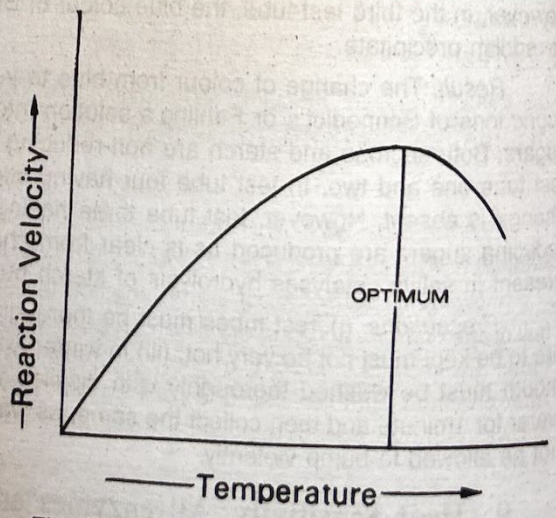


Fig. 9.29. Relation between temperature and enzyme controlled reaction velocity.

and below this temperature. Enzyme becomes inactive below **minimum temperature** and beyond **maximum temperature**. Low temperature preserves the enzymes in the inactive state. Therefore, it is used in preservation of foods inside cold storages. Low temperature present inside cold storages prevents spoilage of food by two methods : (i) Inactivity of enzymes present inside food article and (ii) Nonactivity of microbes because their enzymes also become inactive at low temperature.

High temperature destroys enzymes by causing their denaturation. This occurs at 50°C or so. In between the minimum and maximum temperatures, the reaction velocity doubles for every rise in 10°C (general rule of thumb). A **time factor** appears beyond optimum temperature. Here there is a rise in velocity for a short time followed by a sharp fall.

As opposed to warm blooded or homoeothermal animals (mammals, birds), there are cold blooded or poikilothermal animals (reptiles, amphibians, fishes, invertebrates) whose body temperature rises or falls with that of environmental temperature. These animals cannot live in very hot or very cold environment as enzyme functioning will be impaired. Because of this reason, frog seeks moist shady environment during summer and lies in an inactive form (hibernation) in the deeper layers of the soil during winter.

Experiment 2. Heat destroys activity of enzyme but not that of a catalyst.

Apparatus. Four test tubes, manganese dioxide (MnO_2), water, beaker, spirit lamp, piece of fresh liver or potato, hydrogen peroxide, boiled and cooled piece of liver or potato, boiled and cooled manganese dioxide solution.

Working. Pour 2 ml of H_2O_2 solution is each of the four test tubes. Add a pinch of manganese dioxde in the first test tube, preboiled and cooled 1 ml of manganese dioxide solution in the second, small piece of fresh liver or potato in third and preboiled and cooled piece of liver or potato in the fourth.

All the test tubes are kept at room temperature if it is summer and warm water (kept at about 38°C) if it is winter. Oxygen bubbles are found to come out of solution in the first three test tubes but not in the fourth one.

Result. Manganese dioxide is a catalyst that helps to break hydrogen peroxide into water and oxygen. The catalyst is not affected by heat as it is clear from evolution of oxygen in the second test tube. Fresh liver or potato contains enzymes peroxidase and catalase that help in evolving oxygen from hydrogen peroxide. The enzymes are functional at room temperature (test tube three) but heating destroys their activity (test tube four). Therefore, heat kills enzyme activity but has little effect on the activity of a catalyst.

Precautions : (i) Test tubes must be thoroughly washed with water before use. (ii) Do not perform the experiment without warm water if it is cold outside. (iii) Keep the temperature of warm water to about 38°C. Do not allow it to go beyond 50°C. (iv) Use fresh potato slice or liver piece.

2. **Optimum *p*H**. Every enzyme has an optimum *p*H when it is most effective. A rise or fall in *p*H reduces enzyme activity by changing the degree of ionisation of its side chains. A change in *p*H may also start reverse reaction. Fumarase catalyses fumarate →malate at 6.2 *p*H and reverse at 7.5 *p*H. Most of the intracellular enzymes function near neutral *p*H with the exception of several digestive enzymes which work either in acidic range of *p*H or alkaline, *e.g.*, 2.0 *p*H for pepsin, 8.5 for trypsin.

Experiment 3. *p*H change inhibits enzyme activity.

Apparatus. Two test tubes. 1% starch solution, saliva, dilute HCl, beaker, water, spirit lamp, iodine solution (I +KI).

Working. Pour 2 ml of starch solution in each of the two test tubes. Add 1 ml of fresh saliva in each. A few drops of dilute hydrochloric acid are added to one of the test tubes to make its solution acidic. Both the test tubes are kept for one hour at room temperature if it is summer or in warm water (about 38°C) if it is winter. After one hour both the test tubes are tested for starch by pouring 1 to 2 drops of iodine solution. Test tube one shows negative starch test while test tube two (acidified) develops blue colour showing the presence of starch.

Results. In test tube one, *p*H is nearly that of saliva. The salivary amylase contained in saliva is functional and causes hydrolysis of starch because starch test is negative. In test tube two appearance of blue colour indicates that the starch has not been hydrolysed by enzyme present in saliva. The only difference

in two test tubes is that the solution of the second test tube has been acidified. Therefore, change in pH inhibits enzyme activity.

Precautions : (i) Test tubes must be thoroughly washed and dried. (ii) Before the collection of saliva mouth must be washed and any acidic or alkaline food must not be taken. (iii) Warm water must be used in winter. (iv) Care must be taken that there is no contamination of first test tube by the HCl which is being used for second test tube.

3. **Enzyme Concentration**. The rate of a biochemical reaction rises with the increase in enzyme concentration upto a point called limiting or saturation point (Fig. 9.30). Beyond this, increase in enzyme concentration has little effect.

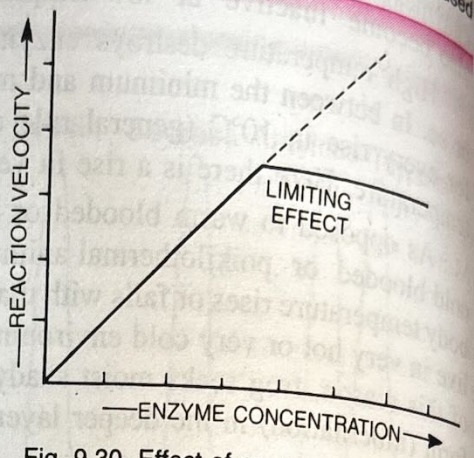


Fig. 9.30. Effect of enzyme concentration on the rate of biochemical reaction.

4. **Product Concentration**. If the products are allowed to remain in the area of the reaction, the rate of forward reaction will fall. Reverse reaction can also start.

5. **Activators**. They increase activity of enzymes (*e.g.*, chloride for salivary amylase), function as cofactors (*e.g.*, K^+, Mn^{2+}) and convert proenzymes to enzyme state. HCl of digestive juice changes proenzyme pepsinogen to enzyme pepsin. Pepsin also possesses **autocatalytic property** as it can also change pepsinogen to pepsin state.

6. **Protein Poisons**. Cyanides, azides, iodoacetate, and salts of heavy metals destroy tertiary structure of enzymes by either combining with cofactor or a group of apoenzyme (—SH group, —COOH).

7. **Radiation Energy**. High energy radiations break hydrogen bonds, ionic bonds, and other weak linkages to destroy enzyme structure.

8. **Substrate Concentration**. Increase in substrate concentration increases the rate of reaction. The enhanced rate is due to two factors : (a) occupation of more and more active sites by the substrate molecules; (b) higher number of collisions between substrate molecules. The rise in velocity is quite high in the beginning but it decreases progressively with the increase in substrate concentration. If a graph is plotted for substrate concentration *versus* reaction velocity, it appears as a hyperbolic curve. A stage is reached where velocity is maximum. It does not increase further by increasing the substrate concentration. At this stage the enzyme molecules become fully saturated and no active site is left free to bind additional substrate molecules. This saturation effect is shown by all enzymes. Because of this Victor Henri (1903) proposed the formation of enzyme–substrate complex as an essential step in enzyme catalysis.

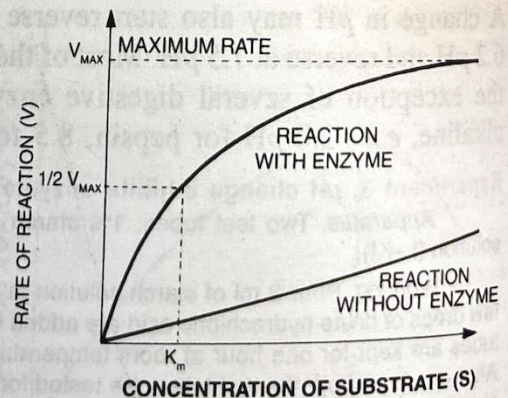


Fig. 9.31. Effect of substrate concentration on reaction velocity with or without enzyme, calculation of maximum velocity and Km value.

Michaelis Constant (Michaelis Menten Constant, Km). It is a mathematical derivation or constant which indicates the substrate concentration at which the chemical reaction catalysed by an enzyme attains half its maximum velocity (Fig. 9.31). Constant was given forth by Leoner

Michaelis and Mand Menten (1913). Km or Michaelis Menten constant generally lies between 10^{-1} to 10^{-6} M. Km indicates affinity of the enzyme for its substrate. A high Km indicates low affinity while a low Km shows strong affinity. If an enzyme acts on more than one substrate it shows different Km values for them. Thus enzyme protease acts on large number of proteins. Its Km value will differ from protein to protein.

Allosteric enzymes do not show a typical Michaelis Menten constant or behaviour. The classical hyperbolic curve is replaced by a sigmoid saturation curve.

Activation Energy

Most of the chemical reactions do not start automatically because the reactant molecules have an **energy barrier** to become reactive. The energy barrier may be on account of : (i) Mutual repulsion due to presence of electrons over their surfaces. (ii) Solvation or holding of reactants in solution form by hydrogen bonds. (iii) Reaction sites of the reactive molecules being small, precise collisions do not occur. Therefore, an external suply of energy is needed for the start of the chemical reaction. It is called **activation energy**. Activation energy increases the kinetic energy of the system and brings about forceful collisions between the reactants. The requirements of activation energy is quite high. For example, acidic hydrolysis of sucrose requires 32000 cal/mole of energy. As already noted about 1000 chemical reactions are taking place in a cell at any time. Activation energy required for such a large number of reactions cannot be provided by living systems. Enzymes lower the activation energy required for a reaction (Fig. 9.32). For example, in the presence of enzyme sucrase or invertase, hydrolysis of sucrose requires an activation energy of 9000 cal/mole (instead of 32,000 cal/mole). This is achieved by four ways: (i) Desolvation or taking the reactants out of solution state. (ii) Establishing weak bonds between reactants and enzyme. It releases energy called **bond energy**. (iii) Bringing the reactant molecules closer in the region of active sites of enzymes. (iv) Development of strain in the bonds of the reactants by electrophilic and nucleophilic attack. (v) Formation of unstable intermediate structural states collectively called transition state. During the transition state the substrate bonds are broken and new bonds are established that transform the substrate molecules into products. (vi) In exothermic reactions, the energy content of the products is lower than that of substrate (Fig. 9.32). It is higher in case of endothermic reactions. However, whether the reaction is endothermic or exothermic, energy is required for pushing the substrate molecules into transition state. The difference in the energy level of substrate (S) and transition state is the activation energy required to start the reaction.

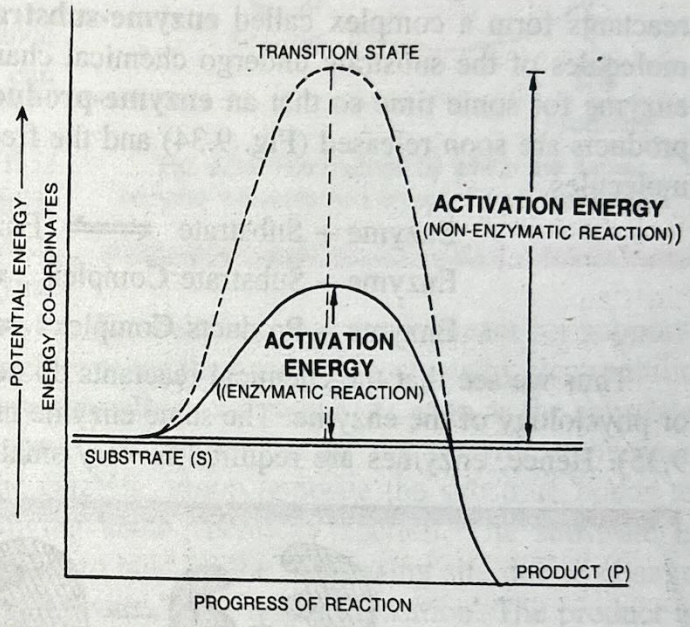


Fig. 9.32. Lowering of activation energy by enzyme in the energy relations of a chemical reaction.

Mode of Enzyme Action

There are two view points by which enzymes are supposed to bring about chemical reaction.

1. **Lock and Key Hypothesis** (Figs. 9.33–9.34). It was put forward by Emil Fischer in 1894. According to this hypothesis, both enzyme and substrate molecules have specific geometrical shapes. In the region of active sites the surface configuration of the enzyme is such as to allow the particular substrate molecules to be held over it. The active sites also contain special groups having $—NH_2$, —COOH, —SH for establishing contact with the substrate molecules. The contact is such that the substrate molecules or reactants come together causing the chemical change. It is similar to the system or lock and key. Just as a lock can be opened by its specific key, a substrate molecule can be acted upon by a particular enzyme. This also explains the specificity of enzyme action.

After coming in contact with the active site of the enzyme, the substrate molecules or reactants form a complex called **enzyme-substrate complex**. In the complexed state the molecules of the substrate undergo chemical change. The products remain attached to the enzyme for some time so that an **enzyme-product complex** is also formed. However, the products are soon released (Fig. 9.34) and the freed enzyme is able to bind more substrate molecules.

$$\text{Enzyme} + \text{Substrate} \rightleftharpoons \text{Enzyme} - \text{Substrate Complex}$$
$$\text{Enzyme} - \text{Substrate Complex} \rightleftharpoons \text{Enzyme} - \text{Products Complex}$$
$$\text{Enzyme} - \text{Products Complex} \rightleftharpoons \text{Enzyme} + \text{Products}$$

Thus we see that the chemical reactants do not cause any alteration in the composition or physiology of the enzyme. The same enzyme molecule can be used again and again (Fig. 9.35). Hence, enzymes are required in very small concentrations.

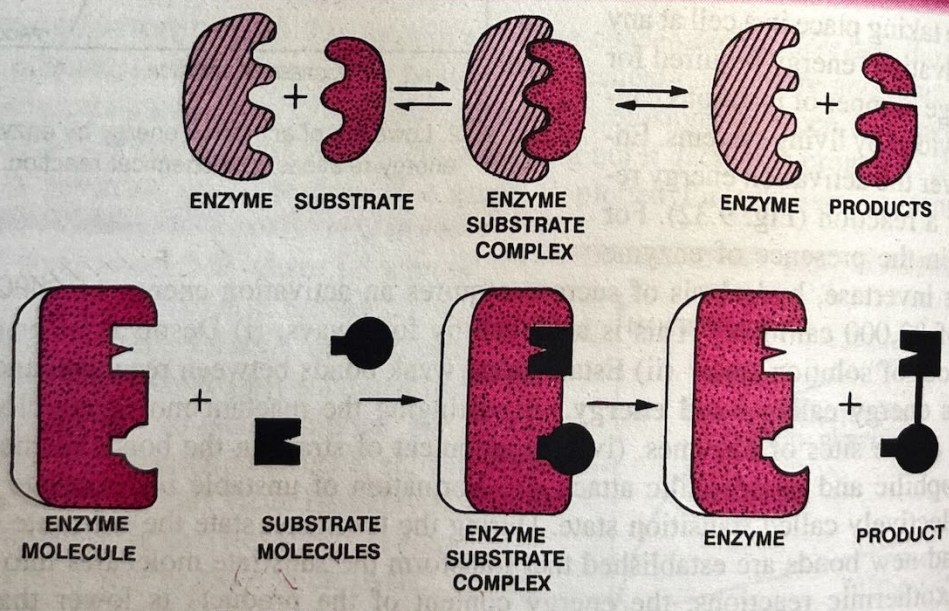


Fig. 9.33. Lock and key theory of enzyme action.
Upper Series – Breakdown Reaction Lower Series – Biosynthetic Reaction

Evidences

1. Blow and Steitz (1970) have found the formation of complex between the enzyme chymotrypsin and its substrate.

2. Keilen and Maun have observed that the absorption spectra of the same enzyme are different in the free state and in the presence of the substrate.

3. The theory explains how a small concentration of enzyme can act upon a large amount of the substrate.

4. Lock and key theory explains how the enzyme remains unaffected at the end of chemical reaction.

5. It is able to predict the increase in the rate of chemical reaction on the addition of more enzyme or substrate.

6. The theory explains how a substance having a structure similar to the substrate can work as competitive inhibitor.

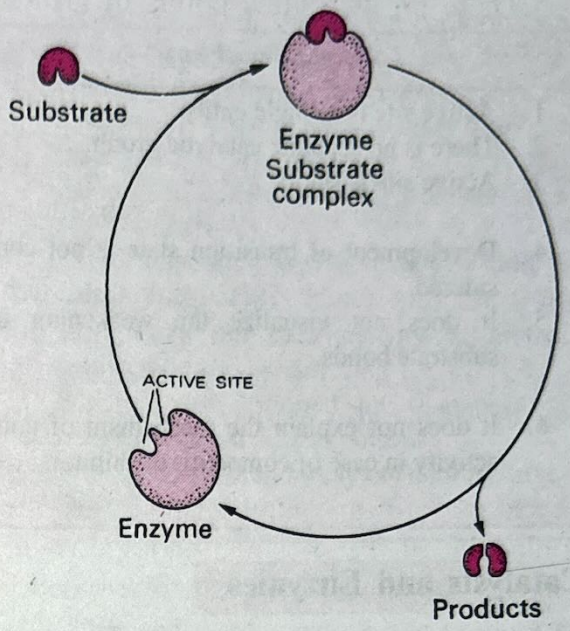


Fig. 9.34. The method by which the same enzyme molecule can be used again and again.

2. **Induced-Fit Theory** (Fig. 9.35). It is modification of lock and key hypothesis which was proposed by Koshland in 1959. According to this theory the active site of the enzyme contains two groups, buttressing and catalytic. The buttressing group is meant for supporting the substrate. The catalytic group is able to weaken the bonds of reactants by electrophilic and nucleophilic forces. The two groups are normally at a distance. As soon as the substrate comes in contact with the buttressing group, the active site of the enzyme undergoes conformational changes so as to bring the catalytic group opposite the substrate bonds to be broken. Catalytic group helps in bringing about chemical reaction. The substrate is converted into product. The product is unable to hold on the buttressing site due to change in its structure and bonds. Buttressing group reverts to its original position. The product is released.

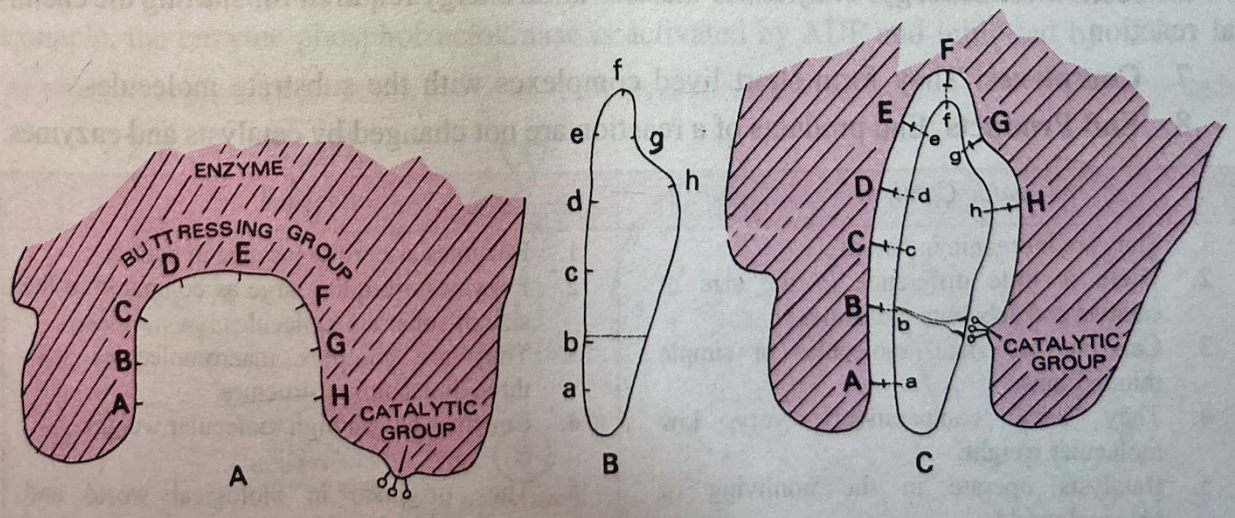


Fig. 9.35. Induced-fit theory of enzyme action. A, active site of enzyme. B, substrate molecule. C, enzyme-substrate complex with conformational changes so as to bring the catalytic group against the substrate bonds to be broken.

Lock and Key Theory	Induced Fit Theory
1. Active site is a single entity.	1. Active site is made of two components.
2. There is no separate catalytic group.	2. A separate catalytic group is visualised.
3. Active site is static.	3. In contact with substratum, the buttressing group undergoes conformational change.
4. Development of transition state is not considered.	4. It considers the development of transition state before the reactants undergo change.
5. It does not visualize the weakening of substrate bonds.	5. Catalytic group is believed to weaken the substrate bonds by nucleophilic and electrophilic attack.
6. It does not explain the mechanism of non-activity in case of competitive inhibitor.	6. It gives a mechanism for nonaction over competitive inhibitor

Catalysts and Enzymes

Catalysts are inorganic substances which increase the rate of chemical reactions without themselves undergoing any change and without modifying the equilibrium of the reactions. Enzymes are similar chemicals which are biological in origin and operate in the biochemical world.

Similarities

1. **Unchanged**. Both catalysts and enzymes remain unchanged chemically and quantitatively at the end of the reaction, so that they can be used over and over again.
2. **Quantity**. They are required in minute quantity as compared to their substrate.
3. **Reversibility**. Reactions controlld by both catalysts and enzymes are theoretically reversible though reversibility is dependent upon different kinetics.
4. **Equilibrium**. They do not change the equilibrium of the reaction.
5. **Reaction Velocity**. Both catalysts and enzymes increase the rate of chemical reaction. They do not initiate the reaction.
6. **Activation Energy**. They lower the activation energy required for starting the chemical reaction.
7. **Complexes**. They form short lived complexes with the substrate molecules.
8. **End Products**. End products of a reaction are not changed by catalysts and enzymes.

Inorganic Catalysts	Enzymes
1. They are inorganic in nature.	1. Enzymes are proteinaceous in nature.
2. There is little difference in the size of catalyst and substrate molecules.	2. Enzymes are quite large as compared to the size of substrate molecules.
3. Catalysts are small molecules or simple mineral ions.	3. They are complex macromolecules with three dimensional structure.
4. They have comparatively very low molecular weight.	4. Enzymes have a high molecular weight.
5. Catalysts operate in the nonliving or physical world.	5. They originate in biological world and mediate biochemical reactions.
6. They are less efficient.	6. They are highly efficient.
7. A catalyst can catalyse diverse reactions.	7. An enzyme catalyses specific reaction of a single or only a few substrates.

8. Their functioning is not controlled by regulator molecules.	8. Enzyme activity can be regulated by specific substances which change the conformation of enzyme molecules.
9. Catalysts are not sensitive to small changes in temperature. They usually operate at high temperature.	9. Enzymes are effective in a narrow range of temperature found in living beings. They get inactivated at low temperatures (10°C and below) and denatured at high temperatures (50°C and above).
10. Catalysts are not influenced by small changes in *p*H.	10. They function effectively only within a narrow range of *p*H.
11. Inorganic catalysts generally work at high temperature and pressure.	11. They carry out their activity at normal temperature and pressure.
12. Protein poisons do not have any adverse effect on catalysts.	12. Enzymes are poisoned by a number of chemicals called protein poisons.
13. Catalysts are not much influenced by short wave radiations.	13. They get denatured by rays of shorter wavelength.

Proenzyme or Zymogen

Proenzyme is the inactive precursor of an enzyme. The term zymogen is often used for inactive precursor of proteolytic enzyme, *e.g.*, pepsinogen for enzyme pepsin. Many enzymes are initially produced in the proenzyme or zymogen state. They become reactive or active enzymes only at a particular *p*H, in the presence of substrate or some special treatment. For example, pepsinogen is changed to active enzyme pepsin in the presence of hydrochloric acid of gastric juice. Thereafter, pepsin has **autocatalytic** effect on further conversion of pepsinogen.

Allosteric Enzymes

They are enzymes which have separate areas for different types of modulators that alter the conformation of the active site so as to make it effective or ineffective (Fig. 9.36). The areas are called **allosteric sites**. The substances which cause change in allosteric sites are known as **modulators**, **allosteric substances** or **effectors**. The latter are of two types—**activators** and **inhibitors**. Allosteric activator binds with an allosteric site in such a way as to make active site operational. Allosteric inhibitor, on the other hand, brings about such a change in the active site that it becomes unable to combine with substrate molecules. For example, the enzyme phosphofructokinase is activated by ADP and inhibited by ATP.

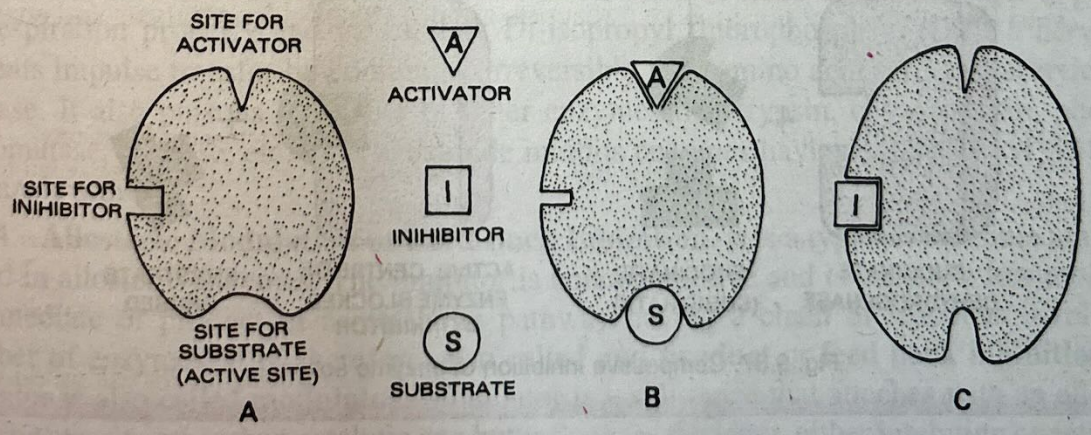


Fig. 9.36. Allosteric enzyme. A, an enzyme with various factors. B, active site becomes functional in the presence of activator. C, inhibitor distorts the enzyme molecule in such a way that the substrate cannot bind to the active site.

Isoenzymes (Isozymes)

At one time it was believed that an organism has only a single enzyme for a given step of a metabolic reaction. It was later discovered that a substrate may be acted upon by a number of variants of an enzyme producing the same product. *The multiple molecular forms of an enzyme occurring in the same organism and having a similar substrate activity are called isoenzymes or isozymes.* Over 100 enzymes are known to have isoenzymes. Thus α-amylase of wheat endosperm has 16 isozymes, lactic dehydrogenase has 5 isoenzymes in man, while alcohol dehydrogenase has 4 isozymes in maize. Isoenzymes differ in activity optima and inhibition. They are thus useful to organism in adapting to varied environmental conditions.

Inhibition of Enzyme Action

Reduction or stoppage of enzyme activity due to presence of adverse conditions or chemicals is called enzyme inhibition. It is of several types. Inhibition can be classified into two (a) Reversible and irreversible (b) Competetive and noncompetitive.

Reversible inhibition is that inhibition which can be overcome by withdrawal of the inhibitor because the effect of the latter is of temporary nature due to blocking of active site or binding to linkages required for maintenance of active site. Dilution and dialysis reduces or eliminates the effect of reversible inhibition. **Irreversible inhibition** is of permanent nature as the enzyme conformation is harmed. Denaturation of enzyme is an example of irreversible inhibition. Heavy metals (*e.g.*, Ag^+, Hg^{2+}, As^+) and iodoacetic acid cause irreversible inhibition by combining with —SH groups and destroying protein structure. Dilution and dialysis have little effect once irreversible inhibition has set in.

Competitive inhibition is caused by swamping of the active sites by a chemical which is similar in structure to the substrate but does not undergo chemical change. Competitive inhibition is usually reversible. **Non-competitive inhibition** is caused by alteration of conformation of the enzyme by a chemical that binds to a site other than the active site. It may be reversible or irreversible. Four common types of enzymes inhibition are as follows :

1. **Protein Denaturation**. Enzyme activity is dependent upon the maintenance of tertiary structure of the protein moiety. The latter is destroyed by several factors like heat, high energy radiaitons and salts of heavy metals.

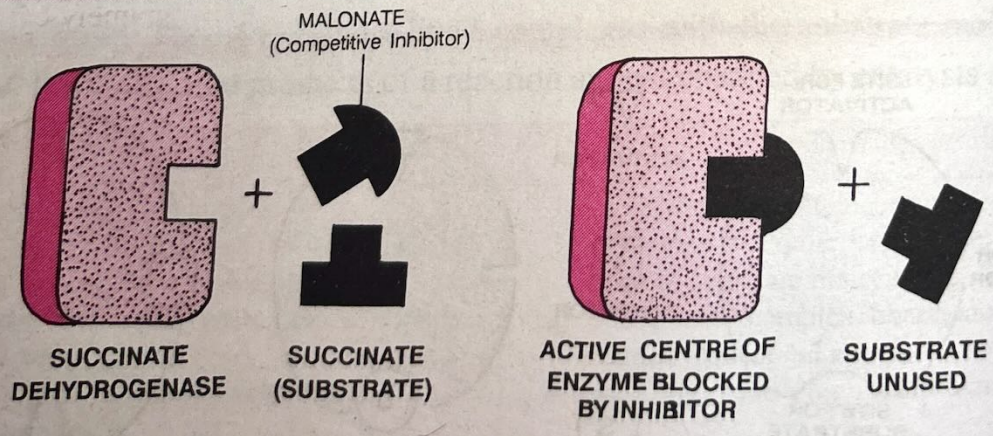


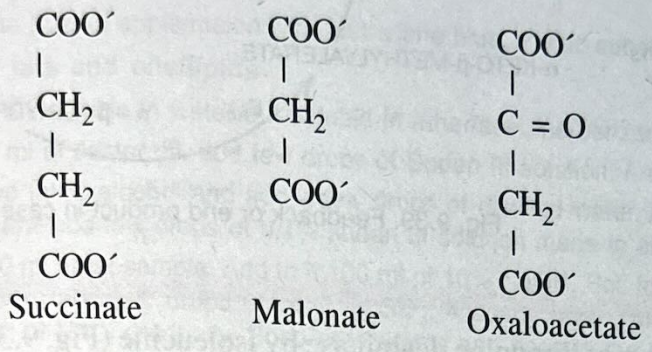
Fig. 9.37. Competitive inhibition of enzyme action.

2. **Competitive Inhibition**. It is the inhibition of enzyme activity by the presence of chemical that competes with the substrate for binding to the active site of the enzyme. The inhibitor chemical is also called **substrate analogue** or **competitive inhibitor**. It resembles

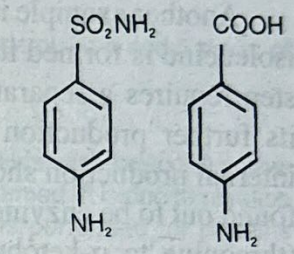
the substrate in structure and gets bound up to the active site of the enzyme without getting transformed by the latter (Fig. 9.37). As a result, the enzyme cannot participate in catalytic change of the substrate. This is similar to the jamming of a lock by a key similar to original one.

Equilibrium constant for inhibitor binding is called K_i. A high K_i reduces enzyme activity while a low K_i allows enzyme activity to continue though at a reduced rate.

Classical example of competitive inhibition is reduction of activity of succinate dehydrogenase by malonate, oxaloacetate and other anions which resemble succinate in their structure.



Competitive inhibition is usually reversible since the addition of more substrate tends to reduce the effect of the inhibitor. The inhibition is important in that (i) It gives evidence for lock and key hypothesis of enzyme action. (ii) Substrate analogues are not metabolised by enzymes. (iii) Control of bacterial pathogens has been effected through competitive inhibition. Sulpha drugs (*e.g.*, sulphanilamide) inhibit the synthesis of folic acid in bacteria by competing with *p*-amino benzoic acid (PABA) for the active site of enzyme. Preformed folic acid is obtained by animal cells. Therefore, sulpha drugs do not harm them.



3. **Non-competitive Inhibition**. It is an irreversible inhibition of enzyme activity by the presence of a substance that has no structural similarity with the substrate. It is of two types, reversible and irreversible. The irreversible noncompetitive inhibitor destroys or combines irreversibility with a functional group of enzyme that is essential for its catalytic function. Cyanide inhibits the activity of cytochrome oxidase by combining with its metallic ions. It has no structural similarity with the substrate of the enzyme, namely cytochrome *c*. Cytochrome oxidase is a respiratory enzyme. In its inhibition, the animal is unable to perform the respiration properly and gets killed. Di-isopropyl fluorophosphate (DFP, a nerve gas) prevents impulse transfer by combining irreversibly with amino acid serine of acetylcholine esterase. It also poisons a number of other enzymes like trypsin, chymotrypsin, phosphoglucomutase, elastase, etc. Iodo-acetamide inhibits enzymes having sulphahydryl (—SH) or imidazole group.

4. **Allosteric Modulation or Feed Back Inhibition**. It is a type of reversible inhibition found in allosteric enzymes. The inhibitor is noncompetitive and is usually a low molecular intermediate or product of a metabolic pathway having a chain of reactions involving a number of enzymes. It is, therefore, also called **end product** or **feed back inhibition**. The inhibitor is also called **modulator**. Modulator is a substance that attaches with an allosteric enzyme at a site other than catalytic one but influences the latter, either inhibiting or activating the same. An example of feed back or allosteric inhibition is stoppage of activity of enzyme hexokinase (glucokinase) by glucose-6-phosphate, the product of reaction catalysed by it (Fig. 9.38).

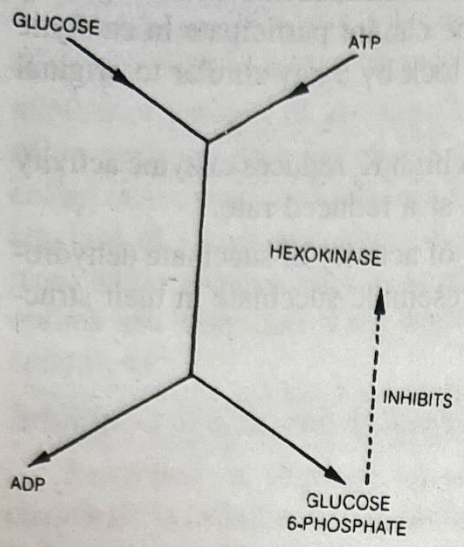


Fig. 9.38. Feedback or allosteric inhibition of hexokinase.

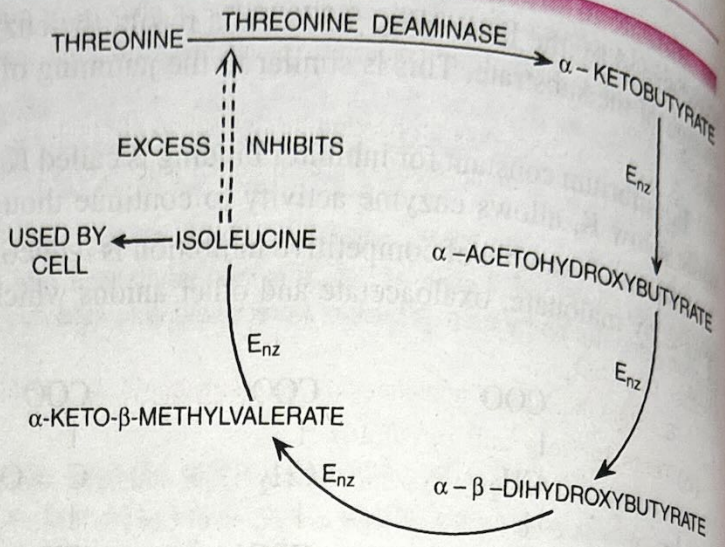


Fig. 9.39. Feedback or end product in case of isoleucine.

Another example is inhibition of threonine deaminase by isoleucine (Fig. 9.3). Amino acid isoleucine is formed in bacterium *Escherichia coli* in a 5-step reaction from threonine. Each step requires a separate enzyme. When isoleucine accumulates beyond a **threshold value**, its further production stops. Isoleucine added to the medium of bacterium also stops its internal production showing that its excess prevents some step of the reaction. The latter was found out to be enzyme threonine deaminase which is involved in the first step of the reaction (threonine to α-ketobutyrate).

Importance. (i) It has a regulatory role on enzyme activity. (ii) Enzyme inhibitors have been used in the study of metabolic pathways. (iii) Some inhibitors are used in controlling pathogenic activity, *e.g.*, sulpha drugs. (iv) Use of inhibitors have shown the mechanism of enzyme action.

Differences between Competitive Inhibition and Allosteric Inhibition

Competitive Inhibition	*Allosteric Inhibition*
1. The inhibitor binds to the active site of enzyme.	1. The inhibitor attaches to an area other than the active site.
2. It does not change conformation of enzyme.	2. Conformation of enzyme is changed.
3. The active site is swamped by inhibitor.	3. Conformation of active site is changed so that substrate cannot combine with it.
4. The inhibitor resembles the substrate in its broad structure.	4. The inhibitor has no structural similarity with the substrate.
5. The inhibitor is not connected by metabolic pathway catalysed by the enzyme.	5. Inhibitor is a product or intermediate of the metabolic pathway connected with that enzyme.
6. It does not have a regulatory function.	6. Allosteric inhibition has a regulatory function as it stops the excess formation of a product.

Qualitative fests for Carbohydrates / Sugars :

(a) Tests for Glucose and Fructose

1. Grape Juice/Fruit Juice contains glucose and fructose. Their presence can be tested by Fehling's

test. Take 5 ml of fruit juice in a test tube. Add an equal quantity of Fehling solution I and II. Boil. A brick red precipitate of cuprous oxide indicates the presence of glucose or fructose in fruit juice. ($Cu^{+2}_{(Blue)} \rightarrow Cu^{+}_{red}$)

2. Fructose gives a red colour with Selivenoff's reagent (resorcinol + conc. HCl) while glucose gives no colouration.

3. Glucose chars only on heating by the action of conc. H_2SO_4 while fructose chars in cold.

4. To 5 cc of Benedict's solution, add 3cc of glucose solution. On boiling, green/red yellow/rust brown precipitate is formed.

5. Sucrose gives negative with Fehling's test. It is first hydrolysed by conc. H_2SO_4. Boil 5cc of sucrose solution with few drops of conc. H_2SO_4. It is then neutralised with NaOH. Boil again and then add Fehling's solution I and II drop by drop. A brick red precipitate is formed due to hydrolysis of sucrose into glucose and fructose by H_2SO_4.

6. Stain the pulp of apple/melon with methylene blue. Pectic substances in cell wall are stained violet.

(b) Tests for fats and oils/lipids

1. Fats are insoluble in water but soluble in ether/acetone/benzene.

2. To few ml of castor oil, add few drops of Sudan III solution. A reddish colour appears.

3. Dissolve fat in alcohol and add a few drops of distilled water. An emulsion of fat in water appears on the surface. Now add few drops of 0.1% sudan III solution made in alcohol. The emulsion turns red.

4. Take 50 ml of fat sample. Add to it 100 ml of 10% NaOH. Boil for 30 minutes. Divide it into two parts A and B. To **A** part, add few drops of conc. H_2SO_4. A soapy layer collects at the surface. To **B** part add saturated solution of NaCl gradually. Soap precipitates and rises to the surface.

5. *Test for glycerol* : Mix 1 ml of 1% $CuSO_4$ solution and 5 drops of the glycerol. To it add 5 drops of 10% NaOH solution. Blue colour is obtained.

(c) Tests for Proteins.

(i) **Xanthoproteic test.** To 5 cc of protein solution, add 2cc of conc. HNO_3. Heat with boiling. A yellow colour appears. Cool it and add excess of 20% NH_4OH or NaOH. Orange colour is formed. It is due to nitration of phenolic groups attached to side chains of aromatic amino acids. This test is performed for proteins containing aromatic amino acids like tyrosine, tryptophan etc.

(ii) Grind protein rich seeds (Gram, Pea) with water to make protein solution. Add **Millons reagent** and heat to boiling. Red colour is formed.

(iii) **Biuret Test.** To 3 ml of protein solution, add 1 ml of 40% NaOH and few drops of 5% $CuSO_4$ solution. Shake and keep for 15 minutes at room temperature. A violet/pink colour appears which gradually changes to blue and purple. Actually Cu^{++} reacts with CONH of proteins and forms a violet coloured complex called biuret ($CONH_2—NH—CONH_2$).

(iv) Beat white of egg with 8 times its volume of H_2O. Filter and perform xanthoproteic test.

(d) Test for Amino Acids.

Ninhydrin test is best to detect amino acids. Most of the amino acids give purple colour with ninhydrin but tyrosine, phenylalanine aspartic acid give blue colour; tryptophan produces olive brown and proline gives yellow colour.

0.2% ninhydrin solution made in 70% alcohol is put on a piece of filter paper and dried. Now put a drop of aqueous solution of amino acids on this dry filter paper. Dry in oven. A blue/violet/purple colour appears due to the reaction of ninhydrin with amino group of amino acid to form Ruhemann's purple compound.

(e) Test for Urine.

Urine is tested for presence of urea, uric acid, creatinine, minerals (Cl, SO_4, Ca, PO_4), sugar and albumin protein.

(i) *Urine for presence of urea* : Urea when heated decomposes with the liberation of ammonia and the formation of biuret. The biuret is dissolved in water and develops a violet colour forming a complex with alkaline $CuSO_4$ solution. Place a small amout of urea crystals in a dry test tube and heat it in a low flame. Urea melts and solidifies. (In case of urine, urine is heated to solidify). Cool the test tube. Add 3 ml of water and shake. Add 1 ml of dil NaOH and 2 drops of 1% $CuSO_4$ solution. Pink colour develops indicating presence of urea.

(ii) For detection of sugar in urine perform Benedicts test. To about 5 ml of Benedicts reagent, add 0.5 ml (8 drops) of urine and boil for 2 minutes. A light green/yellow/brick red precipitate indicates the presence of reducing sugar in urine. The intensity of colour depends upon percentage of sugar in urine.

(iii) *For albumin in urine.* (a) Fill 3/4th of the test tube by urine after filtering it. Heat the upper 1/3rd of

the test tube by a small flame. A turbidity is found on the heated portion of the urine. Add a drop of 33% acetic acid to the urine. Phosphate is dissolved but not the albumin protein. (b) Add a few drops of 30% sulphosalicylic acid to 2 ml of clear filtered urine. A turbidity indicates the presence of albumin.

ADDITIONAL INFORMATION

- **Fritz Lipmann** (1941). Father of ATP cycle.
- **Melting Points of Fatty Acids**

Palmitic Acid	63.1°C
Oleic Acid	13.4°C
Stearic Acid	69.6°C
Linoleic Acid	– 5°C
Arachidic Acid	76.5°C
Linolenic Acid	– 11°C
Arachidonic Acid	– 49.5°C

- **Glycosidic Bond**. Bond between a sugar and another molecule through an intervening oxygen (O-glycosidic) or nitrogen (N-glycosidic) atom.
- **Magic 20**. The twenty amino acids which get arranged in a variety of ways to produce innumerable types of proteins.
- **Most Abundant Components of Cell/Tissue/Organisms**. Water (65—70% in human body), proteins (9%, maximum amongst organic components), carbohydrates (2 — 3%), lipids (1.5 — 2.0%) and nucleic acid (1 — 2%). In plants, carbohydrate content is higher due to presence in cell wall.
- **Most Abundant Mineral**. In vertebrate body the most abundant mineral is calcium (2%) followed by Phosphorus (1%).
- **Most Abundant Mineral in Body Fluids**. Na^+ followed by Cl'.
- **Most Abundant Mineral in Cellular Pool**. K^+ followed by phosphate.
- **Milk**. Human milk contains maximum amount of lactose (milk sugar), all the essential amino acids in proper proportions, all the essential fatty acids but only in low quantity.
- **DNA**. The term was coined by Zacharis.
- **DNA Content** (c-Value). 1 picogram (10^{-12} gm) DNA is roughly 31 cm long. A human cell contains 5.6 pg of DNA, about 2m in length. Length of DNA is 3m in Pea.
- **Haemoglobin**. C_{3032} H_{4816} O_{872} N_{780} S_8 Fe_4 (molecular weight 66552).
- **Sanger** (1953). The first to decipher the sequence of amino acids in a protein (bovine insulin).
- **Polypeptide Coiling**. α-helix has a pitch of 5.4Å while β-pleated shows a pitch of 7.0 Å. Each turn of α-helix has 3.5 amino acids.
- **Artificial Silk**. It is a polysaccharide.
- **Abundant Organic Molecules**. Most abundant is cellulose followed by chitin. Most abundant protein is **Rubisco** (ribulose biphosphate carboxylase).
- **Enzymes in Detergents**. Proteases are added in detergents used for washing clothes and amylase in detergents used for dish washing.
- **Baby Foods**. Trypsin is added to partially pre-digest the foods.
- **Enzymology**. Branch of science dealing with enzymes, their reactions and functions.
- **Allozymes**. Similar enzymes which are produced by different genes.
- **Constitutive Enzymes**. The enzymes are always present because of their requirement for a vital process, *e.g.*, glycolysis.
- **Repressible Enzymes**. Enzymes normally present but are repressed when specific chemical or product is present, *e.g.*, glucokinase.
- **Inducible Enzymes**. Enzymes formed in response to presence of its substrate, *e.g.*, lactase.
- **Sumner**. Found that enzymes are proteinaceous (1926). He was the first to crystallise an enzyme, urease from Jack Bean (*Canavalia ensiformis*).
- **Genomic or Genetic RNA**. Discovered by Conrat (1957).
- **rDNA**. DNA present in nucleolar organiser region which is specialized to transcribe rRNAs. It has a number of repeat units.
- **Satellite DNA**. It is DNA with repetitive sequences which being heavier or lighter forms a separate fraction during ultracentrifugation. Satellite DNA occurs in heterochormatic regions as well as other parts.
- **Highly Repetitive DNA**. It consists of short simple sequences which are repeated hundreds of thousands times (Skinner, 1978). Satellite DNA is highly repetitive DNA, *e.g.*, pericentromeric regions of chromo-

somes. Heterochromatic region of Y-chromosome has special satellite DNA found only in male cells.

- **Moderately Repetitive DNA**. They are repeated a few hundred times. Centromere, telomere, and ends of transposons are moderately repetitive DNA.
- **Minisatellite Sequences**(Jeffreys, 1985). They are hypervariable repeat sequences of DNA, 11-60 base pairs and flanked by conserved restriction sites.
- **Microsatellite Sequences.** They are simple sequence repeats with1-6 base pairs and flanked by conserved sequences.
- **Mini-Microsatellite DNA.** Portions of DNA that use small repeats and unique to each person. Used for DNA finger printing.
- **Artificial Gene.** First artificial gene was synthesised by Khorana *et al* (1968). It was alanine-tRNA gene with 77 base pairs. The gene, however, did not function in living system. Their second artifical and functional gene was tyrosine-tRNA gene with 207 base pairs (Khorana et al, 1979).
- **Khorana** *et al* (1968). Synthesised alanine *t*RNA having 77 nucleotides. It was non-functional.
- **Khorana** *et al* (1979). Synthesised tyrosine *t*RNA having 207 nucleotides. It was functional.

NCERT TEXTBOOK QUESTIONS WITH ANSWERS

1. What are macromolecules ? Give examples.

✓ Macromolecules are large sized chemicals which get separated in acid insoluble fraction using trichloroacetic acid. They are polymers with a molecular mass of 10,000 daltons or above, *e.g.,* polysaccharides, proteins, nucleic acids. Lipids are neither polymers nor have a high molecular weight but being acid soluble they are included amongst macromolecules.

2. Illustrate a glycosidic, peptide and phosphodiester bond.

✓ (i) **Glycosidic Bond.** It is a bond formed during dehydrate synthesis of polysaccharide with one monosaccharide providing hydroxyl (– OH) and other providing hydrogen (– H) to form a molecule of water (HOH). The bond is generally – COC – or – C.N.C – $C_5H_{10}O_5$. HCOH + OH.$CHO_5H_{10}C_5$ → $C_5H_{10}O_5$.HCOCH.$O_5H_{10}C_5$ + HO

(ii) **Peptide Bond.** It is a bond formed during dehydrate synthesis of a peptide with one amino acid providing hydroxyl (– OH) and other hydrogen (– H) to form a molecule of water (HOH). The bond consists of – CONH.

$$H_2N \cdot \underset{\displaystyle R}{\underset{|}{CH}} \cdot COOH + HN\,H - \underset{\displaystyle R}{\underset{|}{CH}}\,COOH \rightarrow H_2N - \underset{\displaystyle R}{\underset{|}{CH}} \cdot CO \cdot NH - \underset{\displaystyle R}{\underset{|}{CH}} \cdot COOH + H_2O$$

(iii) **Phosphodiester Bond.** It is a double ester bond formed during dehydrate synthesis in the reaction between a phosphoric acid ($HOHPO_2OH$) and two pentose sugars. The bond is –O. HPO_2.O– $C_4H_7O_3$. CH_2OH + $HOHPO_2$. OH + $HOCH_2O_3H_7C_4$ → $C_4H_7O_3.CH_2O$. HPO_2O $CH_2O_3H_7O_4$ + 2 H_2O

3. What is meant by tertiary structure of proteins ?

✓ **Tertiary Structure.** It is a structure formed by folding of secondarily coiled polypeptide, α -helix and β -pleated, in such a way as to bring the functional side groups to the surface and inactive side groups in the interior.

4. Find and write down structures of 10 interesting small molecular biomolecules. Find if there is any industry which manufactures the components by isolation. Find out who are the buyers.

✓ The 10 interesting small molecular biomolecules are— ethyl alcohol, tartaric acid, lactic acid, citric acid, amino acid, trypsin, sucrose, glucose, gluconic acid and amylose. Further, consult any book on Industrial Biochemicals/Biotechnology.

5. Proteins have primary structure. If you are given a method to know which amino acid is at either of the two termini (ends) of a protein, can you connect this information to purity or homogenesity of a protein?

✓ Simply knowing the two amino acids at the two ends of a protein cannot help in designating a protein as pure or homogeneous. It is because a protein is made of numerous amino acids. A difference of only one amino acid any where will made the protein different from the other. Only by sequencing the complete protein by Edman or Sanger's technique can tell us about purity of a protein.

25. Give technical names of vitamins B_1, B_2, A and K.
✓ B_1 — Thiamine, B_2 — Riboflavin, A— Retinol (= Auxerophthol), K — Phylloquinone.

26. Write a note on cellulose.
✓ It is fibrous homopolysaccharide of high tensile strength which forms structural elements of ce wall in all plants, some fungi and protists. It consists of a linear unbranched chain of 6000 or more 1-4 linked glucose residues. Some 2000 molecules of cellulose occur in a bundle to form a structur element called microfibril.

27. Name two structural polysaccharides which are found in fungi.
✓ Structural polysaccharides found in fungi are chitin and cellulose. (i) **Cellulose**. It occurs in a few fungi either alone (*e.g., Phytophthora*) or alongwith chitin. Cellulose is a homopolysaccharide of β 1 4 glucose residues. (ii) **Chitin** (Fugus Cellulose). In most fungi the structural polysaccharide of wa is chitin or fungus cellulose. It is a heteropolysaccharide of acetyl glucosamine residues.

28. Arrange the following carbohydrates in the order of increasing complexity of chemical structures fructose, starch, oligosaccharide, maltose, triose.
✓ Triose, Fructose, Maltose, Oligosaccharides, Starch.

29. What do you understand by the antiparallel arrangement of DNA strands?
✓ The two DNA chains are **antiparallel** that is, they run parallel but in opposite directions. In one chain the direction is 5' →3' while in the opposite one it is 3' →5' (Fig. 9.23). The two chains are held together by hydrogen bonds between their bases.

30. What is anabolic steroid ? Explain its usefulness.

31. What is competitive inhibition ? Give an example.

32. How are enzymes able to enhance the rate of reaction ?

33. Why are starch and glycogen more suitable than glucose as storage products ?

34. Distinguish between prosthetic group and co-factors.

35. Which of the nucleotide compositions will be possible, if DNA is double stranded ?
(a) All A; (b) Only A and T; (c) Only C and T; (d) Only A and G; (e) only A, G and T.

36. List three main differences between DNA and RNA ?

37. Which molecule bears codons and which molecule anticodons ?

Three Mark Questions (Short Answer Type)

38. What is the function of ATP in cell metabolism ? Explain with the aid of diagram, how its structure makes it possbile.

39. What is cholesterol ? Give its functions and disorders produced by it in the human body.

40. What are phospholipids ? Briefly discuss their structure, properties and functions.

41. Why do phospholipids produce bilayer in contact with water ?

42. What role do minerals play in maintaining the structure of animals.

43. Although all proteins are made of the same amino acids, explain how your proteins may be different from those of a dog.

44. What is pleated sheet organisation ? Can there be quarternary structure ?

45. (a) Name the chemical that cements animal cells.
(b) Name two contractile proteins.
(c) What is the difference between RNA and DNA in their nitrogen bases.

46. List the nature, source(s), monosaccharide unit and function of the following polysaccharides : starch, glycogen and cellulose.

47. (a) What is an enzyme ?
(b) Give an example of apoenzyme.
(c) Give an example of coenzyme.

48. (a) What do you understand by the term apoenzyme ?
(b) Distinguish between isoenzyme and coenzyme.
(c) Distinguish between apoenzyme and coenzyme.
(d) Differentiate between enzyme and isoenzyme.

49. List three important properties of enzymes.

50. What does an enzyme do in terms of energy requirement of a reaction ? What would happen if the enzyme did not play this role ?

51. Define the terms (a) Active site (b) Turn over number (c) Non-competitive Inhibition (d) Isoenzyme (e) Zygmogen (f) Activation energy.
52. Describe the structure of phospholipids. How are they arranged in the cell membrane ?
53. Write short note on (i) steroids (ii) wax
54. How does the substrate concentration affect the velocity of enzyme reaction ?
55. What is competitive inhibition ? Compare it with allosteric inhibition.
56. Why allosteric inhibition is called feedback inhibition ? Explain.
57. How do primary metabolites differ from secondary metabolites?
58. Given below is the transcribed strand of the DNA duplex : 3′ – TAC CGA TCC GAG CTG – 5′
    (a) Draw the complementary DNA polynucleotide chain
    (b) Construct the RNA molecule, which will be transcribed.
59. (a) How are the two strands of DNA helix held together ?
    (b) In chemical terms what are DNA and RNA ?

Five Marks Questions (Long Answer Type)

60. (a) What will happen if starch amylase mixture is incubated at 0°C for half an hour ?
    (b) Describe the role of temperature, *p*H and substrate affecting enzymatic activity.
    (c) What would happen when salivary amylase that acts specifically on starch enter the stomach and mixes with gastric juice ?
61. (a) What are enzymes ?
    (b) Discuss the mechanism of enzyme action.
62. Write a note on the types and structure of proteins.
63. Briefly discuss the structure and functions of DNA.
64. What are carbohydrates ? Define small carbohydrates and discuss their types.
65. Enumerate the functions of small carbohydrates in the living organisms.
66. What are lipids ? Give their types.
67. What are amino acids ? Discuss their types and functions.
68. Describe functions of polysaccharides in living organisms.
69. Iliustrate the lock and key hypothesis of enzyme action.
70. What are secondary metabolites ? Name five important secondary metabolitesm and their role in plant life.
71. Write a note on phenolics, flavonoids and tannins.

Multiple Choice Questions (With Answers)

(1) DNA differs from RNA in having (a) Uracil (b) Thymine (c) Adenine (d) Guanine. (*RPMT 1996*)

(2) Double helix model of DNA was proposed by (a) Nirenberg, (b) Kornberg, (c) Holley and Nirenberg, (d) Watson and Crick. (*APMEE 1996, RPMT 1996*)

(3) On hydrolysis, nucleoside does not yield (a) Phosphoric acid (b) Pentose sugar (c) Purine (d) Pyrimidine. (*DPMT 1996*)

(4) Proteins are a polymerisation product of (a) Monosaccharides (b) Muramic acid (c) Amino acids (d) All the above. (*CPMT 1996*)

(5) The amino acids which are not synthesized in our body are called (a) Nonessential (b) Deaminated (c) Proteinaceous (d) Essential (*UPCPMT 1996*)

(6) No cell could live without (a) Phytochrome (b) Enzymes (c) Chloroplast (d) Protein.(*MPPMT 1997*)

(7) Fibrous protein is (a) Haemoglobin (b) Hordein (c) Globulin (d) Collagen. (*Pb. PMT 1997*)

(8) Polymer of α-D glucose is (a) Cellulose (b) Glycogen (c) Inulin (d) Chitin. (*AMU 1997*)

(9) Joining repeating units for formation of a macromolecule is called (a) polymorphism (b) condensation (c) aggregation (d) polymerisation. (*Pb. PMT 1997*)

(10) The enzyme which combines with a nonprotein prosthetic group to form a functional enzyme is called (a) Apoenzyme (b) Holoenzyme (c) Coenzyme (d) Proenzyme. (*AIIMS 1996, AFMC 1997*)

(11) Enzyme amylase belongs to category (a) Oxidoreductases (b) Transferases (c) Hydrolases (d) Isomerases. (*CET Chd 1997*)

(12) Enzyme involved in transfer of electrons belongs to (a) Hydrolase (b) Desmolase (c) Transaminase (d) Dehydrogenase. (*MPPMT 1998*)

(13) Cellulose is (a) branched chain with α -1, 6 and β -1, 4 bonds (b) unbranched chain with α -1, 6 bonds (c) unbranched chain with β -1, 4 bonds (d) branched chain with α -1, 4 and β -1, 6 bonds.

(14) Energy currency of cell is (a) ADP (b) ATP (c) GDP (d) NAD. *(Har PMT 1998)*

(15) Phospholipids are (a) Amphipathic (b) Amphibolic (c) Hydrophobic (d) Hydrophilic. *(DPMT 1999)*

(16) Distance between two adjacent nitrogen bases of DNA is (a) 2.4 Å (b) 3.4 Å (c) 24 Å (d) 34 Å.

(17) Base pairs found in one turn of DNA spiral are (a) 12 (b) 11 (c) 10 (d) 8. *(Pb 1999, MP 2000)*

(18) Most abundant enzyme is (a) Rubisco (b) Catalase (c) Invertase (d) Nitrogenase. *(CBSE 1999)*

(19) Induced fit theory of enzyme activity was given by (a) Fischer (b) Summer (c) Northrop (d) Kcshland. *(Pb PMT 1999)*

(20) Enzymes increase the rate of biochemical reaction through (a) Changing equilibrium (b) Lowering activation energy (c) Forming reactant-product complex (d) Forming enzyme-product complex.

(21) DNA and RNA resemble each other in possessing (a) Similar pyrimidine bases (b) Similar sugars (c) Ability to replicate (d) Polymers of nucleotides. *(CBSE 2000)*

(22) Number of fatty acid residues found in one molecule of fat is (a) 1 (b) 2 (c) 3 (d) 4. *(BHU 2000)*

(23) ATP is (a) Purine base (b) Nucleoside (c) Nucleotide (d) Nucleosome. *(CBSE 2000)*

(24) Which one is the basic amino acid (a) Lysine (b) Leucine (c) Aspartic acid (d) Methionine.

(25) Fehling's solution is used to detect (a) Sucrose (b) Glucose (c) Fat (d) Starch. *(CBSE 2001)*

(26) The most abundant organic molecule present on earth is (a) Protein (b) Lipid (c) Cellulose (d) Steroids. *(CBSE 2001)*

(27) Km value of enzyme is substrate concentration (a) One fourth V_{max} (b) V_{max} (c) Twice V_{max} (d) Half V_{max}.

(28) Nicotinamide can be synthesised in human body from (a) Fructose (b) Lactose (c) Tyrosine (d) Tryptophan. *(AIIMS 2002)*

(29) Collagen is (a) Lipid (b) Fibrous protein (c) Carbohydrate (d) Globular protein. *(CBSE 2002)*

(30) Element located in centre of porphyrin ring of chlorophyll is (a) Potassium (b) Manganese (c) Magnesium (d) Calcium. *(CBSE 2003)*

(31) In which of the form is glucose stored in liver (a) Cellulose (b) Glycogen (c) Starch (d) Sucrose. *(AFMC 2003)*

(32) Chitin occurs in cell wall of (a) Bacteria (b) Algae (c) Fungi (d) Yeast. *(Orissa 2004)*

(33) Match the columns and find out the correct combination

Column I		Column II	
(A)	Starch	(P)	Protein synthesis
(B)	Haemoglobin	(Q)	Sex hormone
(C)	RNA	(R)	Storage product
(D)	Steroid	(S)	Transport of gases

(a) A—R, B — P, C —S, D — Q (b) A—R, B—S, C—P, D—Q

(c) A—S, B—R, C—P, D—Q (d) A—R, B—S, C—Q, D—P *(Karnataka 2004)*

) Organic compounds produced by some plants of certain genera and families which are not in mainstream of metabolism and appear to have no direct function in growth and development of plants are called (a) natural products (b) secondary plant products (c) secondary metabolites (d) all of above.

Which of the following function are attributed to different secondary metabolites in plants? (a) plant defenses against herbivores and pathogens (b) as agents of plant-plant competition (c) to attract animals for pollination and seed dispersals (d) all of above

Secondary plant products are of great use in commerce in making (a) drugs (b) poisons and insecticides (c) flavours and perfumes (d) all of above

Monoterpene esters called pyrethoids found in flowers and leaves of chrysanthemum are used on commercial is making (a) perfumes (b) insecticides (c) drugs (d) all of above

Steroids are (a) Tetraterpenes (b) Polyterpenes (c) triterpenes and their derivative. (d) phenolic compounds

A saponin called Yamogenin from Yam plant, *Dioscorea* is used as (a) insecticide (b) perfume (c) insect repellent (d) Oral contraceptive

Alkaloids in plants function as (a) growth hormone (b) provide protection against predators (c) attract animals for pollination (d) all the above.

(41) Length of DNA with 23 base pairs is (a) 78.4 Å (b) 78.2 Å (c) 78 Å (d) 74.8 Å. *(Kerala 2004)*

(42) The ratio constant for a species is (a) A + G / C + T (b) T+ C /G + A (c) A + C / T + G (d) G+ C / A +T. *(CBSE 2004)*

(43) Bond found between two molecules of carbohydrate is (a) Amide (b) Phosphodiester (c) Glycosidic (d) Hydrogen bond. *(CPMT 2005)*

(44) An amino acid not optically active is (a) Glycine (b) Valine (c) Isoleucine (d) Leucine. *(BHU 2005)*

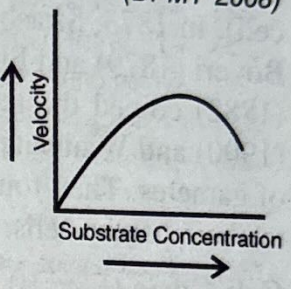
(45) An organic substance essential for activity of an enzyme is (a) Apoenzyme (b) Holoenzyme (c) Isoenzyme (d) Coenzyme. *(CBSE 2006, AFMC 2008)*

(46) Nonprotein part of enzyme is (a) catalytic agent (b) cofactor (c) active site (d) apoenzyme. *(HP PMT 2007)*

(47) 98% of living body is formed of six elements — carbon, hydrogen, nitrogen, oxygen and (a) S and Mg (b) Mg and Na (c) P and S (d) Ca and P. *(CBSE 2007)*

(48) Metal ion required for functioning of enzyme is (a) cofactor (b) coenzyme (c) prosthetic group (d) holoenzyme. *(DPMT 2008)*

(49) Given graph shows effect of substrate concentration on rate of reaction of enzyme phosphatase. What does it indicate ? (a) Rate is proportional to substrate concentration (b) Presence of an enzyme inhibitor (c) Formation of E – S complex (d) At high substrate concentration *p*H changes. *(AIIMS 2008)*



(50) A competitive inhibitor of succinate dehydrogenase is (a) Malate (b) Oxaloacetate (c) α -ketoglutarate (d) Malonate. *(CBSE 2008)*

(51) Properties of starch useful for making it storage material are (i) easily translocated (ii) chemically non reactive (iii) easily digestible (iv) Osmotically inactive (v) synthesized during photosynthesis. (a) (i) and (v) (b) (ii) and (iii) (c) (ii) and (iv) (d) (i), (iii) and (v). *(CBSE 2008)*

(52) Enzymes catalysing removal of group and formation of double bonds are (a) Ligases (b) Lyases (c) Transferases (d) Oxidoreductases. *(CET Chd. 2009)*

(53) An essential fatty acid is (a) Arachidonic acid (b) Arachidic acid (c) Palmitic acid (d) Stearic acid. *(AFMC 2009)*

(54) Class of enzymes contained in lysosomes is (a) Lyases (b) ligases (c) hydrolases (d) transferases. *(AFMC 2010)*

(55) Formation of both peptide and glycosidic bonds involves (a) esterification (b) hydration (c) dehydration (d) acidification. *(DPMT 2010)*

(56) Protein quality depends upon (a) Coagulability (b) Isoelectric point (c) Essential amino acids (d) Quaternary structure. *(Wardha 2011)*

(57) Prostaglandins are (a) Simple proteins (b) Conjugated proteins (c) Saturated fatty acids (d) Unsaturated fatty acids. *(AFMC 2011)*

(58) Important site for formation of glycoproteins and glycolipids is (a) vacuole (b) plastids (c) lysosome (d) Golgi apparatus. *(CBSE 2011)*

ANSWERS

Multiple Choice Questions

(1) —b	(2) —d	(3) —a	(4) —c	(5) —d	(6) —b	(7) —d	(8) —b	(9) —d	(10) —b
(11) —c	(12) —d	(13) —c	(14) —b	(15) —a	(16) —b	(17) —c	(18) —a	(19) —d	(20) —b
(21) —d	(22) —c	(23) —c	(24) —a	(25) —b	(26) —c	(27) —d	(28) —d	(29) —b	(30) —c
(31) —b	(32) —c	(33) —a	(34) —b	(35) —d	(36) —d	(37) —b	(38) —c	(39) —d	(40) —b
(41) —b	(42) —d	(43) —c	(44) —a	(45) —d	(46) —b	(47) —c	(48) —a	(49) —b	(50) —d
(51) —c	(52) —b	(53) —a	(54) —c	(55) —c	(56) —c	(57) —d	(58) —d		